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A NEW TECHNIQUE FOR SAMPLING AND ASSESSING AERIAL SPRAY DEPOSITS^{1, 2}

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ABSTRACT

Printflex paper cards have been found to be a satisfactory simple surface for collecting samples of aerial sprays. The spread factor for an oil solution of an insecticide on Printflex paper is not constant for the various droplet sizes. Spread factors for dyed and undyed solutions of DDT in oil, on dyed and undyed paper, range from 3.6 to 6.2 for corresponding stains produced by droplets in the range 60 to 600 microns. A new rapid photographic method of quantitative assessment of the deposit on Printflex cards is described. The method eliminates the use of film for the process by employing a high contrast light weight photographic paper. The process provides a permanent record of the stains, enlarged 3.5 X, with a suitable calibration grid recorded on the print. The assessment can be carried out without any further optical aids. A statistical analysis of the accuracy of the assessment of deposits on the cards, using the photographic method, has established that density of deposit, number of droplets per unit area, and mass median diameters of the spray sample can be determined with a high degree of accuracy by these methods. Comparisons with other sampling and assessment procedures were carried out on a field trial scale and under field trial conditions.

INTRODUCTION

In the last few years it has become increasingly apparent to entomologists involved in applied insecticide research, and to other scientists engaged in research on substances regulating plant growth, that the physical properties of deposits of insecticides and herbicides have a direct bearing on the resulting biological effect. The recently completed investigations of the relation between the physical properties of a DDT spray and its toxicity to larvae of the spruce budworm (*Choristoneura fumiferana* Clem.) (4) have again adequately demonstrated the necessity of having precise knowledge of the density of the deposit, the droplet size spectrum, and an index of the degree of area coverage achieved. These factors have also assumed importance in current studies of the bio-physical relationships involved in the chemical control of biting flies. The design of aerial dispersal equipment for a specific requirement may evolve from an empirical, costly trial-and-error method or preferably from a more rational

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study of the desirable physical and chemical properties of the airborne or deposited segments of the spray. In contrast to these requirements for precise measurement for research purposes, rapid methods of characterizing deposits in a gross qualitative fashion are often required for use on large scale control operations. These methods can only be of value after the required parameters of effectiveness have been established.

Until recently the only field methods available for sampling spray deposits involved the use of pressed filter paper coated cards, known as "jump cards", which contained paper fibres of uniform size, various types of oleophobic coatings on glass slides and sensitized papers. The jump cards were not satisfactory for general use since the lower limit of the stain that can be measured accurately is limited to that produced by a droplet approximately 150 microns in diameter. Oleophobic coatings (3) were found to be unsatisfactory, due to an unknown variable factor of evaporation, and the complications involved in recalibrating spread factors each time the experimental formulation was changed. The magnesium oxide coatings developed by May (6) and the absolute method developed by Hurtig and Perry (5) are satisfactory for laboratory use, but have been found to be too delicate for general field use. While satisfactory sensitized papers for qualitative checking of swath width have been developed for use with water sprays, no sensitized paper has as yet been developed that is satisfactory for field use with oil solutions. Elliott and Davis (2) (1) have developed a pre-dyed paper card which has proven useful for determining whether or not a block of forest has received aeroplane spray treatment. They claim that visual comparison of the deposit on these cards with the deposit on a series of standard cards provides a satisfactory index of the level of spray coverage. With added refinements, this method can possibly be brought to the desired quantitative basis. In its present state, it does not supply the type of data required to establish the parameters necessary for current investigations of the biophysical relationships which determine the efficacy of insecticidal airsprays. In this report a method is described for establishing the true relation between drop size and the spread factor of the resulting stain on undyed Printflex paper.

The process of assessing spray deposits is time-consuming and tiresome. The amount of field experimentation possible is frequently limited by the size of staff available to carry out the assessments. Visual assessment, as employed by Davis and Elliott (1), is not satisfactory. Therefore, in the past, various methods of microscopic examination, enlargement and projection have been employed, involving eye-strain and fatigue, and resulting in a lowering of the accuracy of the assessment. In order to overcome these difficulties, a simple photographic method has been developed which allows the rapid production of enlarged replicas of the spray deposit samples. The photographic method permits the production of a large amount of accurate data with a small staff and a minimum of equipment and space.

The accuracy of data obtained by employing the photographic method as an aid in the assessment of a large volume of field samples has been compared with the accuracy of similar data for the same samples obtained by other assessment methods.

PRINTFLEX PAPER AS A SAMPLING SURFACE

Description of Printflex Paper

The most satisfactory surface for spray sampling was found to be the undyed Printflex paper card (2). This card is sized with casein and the finish is described as "satin white". The finish consists of a mixture of calcium sulphate and calcium carbonate.

Calibration of Spread Factor

1. Materials

Experience has demonstrated that for experimental spray applications and calibration of aircraft spray swath widths, etc., it is more economical of time and effort to add a dyed insecticide concentrate to the spray tank for subsequent dilution than to uniformly pre-dye and dry the cards. In addition, the clarity of the stain and the minimum size of stain produced by a dyed droplet on a white background are superior to the irregular stain produced on pre-dyed cards. In these experiments the oil solvents and carriers used were a mixture of methylated naphthalenes and fuel oil, fuel oil alone, kerosene, and xylene-kerosene mixtures, all with or without a wide range of organic insecticides. These included DDT, pyrethrum concentrates, parathion, endrin, and a range of pyrethrum synergists including piperonyl butoxide and sulfoxide. Williams' Red and du Pont Oil Red dyes were used.

The solutions used for obtaining the calibration data presented in this report were:

- (i) 10 per cent (w/w) technical DDT in a mixture of Velsicol AR-50 and fuel oil (3 : 7 w/w) containing approximately 0.5 per cent of Williams' Red dye. Density 0.90 g./ml.
- (ii) 5 per cent (w/w) technical DDT in a mixture of Velsicol AR-50 and fuel oil (1 : 7 w/w) containing approximately 0.5 per cent Williams' Red dye. Density 0.86 g./ml.

The solutions were filtered before use to remove any undissolved materials or inert dye fillers.

2. Procedure

For the purpose of calibrating spread factor, samples of spray solutions containing the insecticide, solvent and diluent to be tested were applied as droplets of constant predetermined size, produced by means of the vibrating finger apparatus described by Rayner and Hurtig (7). The size of droplets that could be produced by this apparatus was variable, and it was therefore possible to study the spread factor over a range of stains from 218 to 2950 microns in diameter, produced by droplets ranging from approximately 60 to 550 microns in diameter. The lower limit of accurate detection and measurement attempted to date has been 218 microns diameter stains produced by 59 microns diameter droplets. Further refinements in the production and measurement of smaller droplets may extend the range of calibration to lower limits.

Two methods of measuring the true droplet diameter were employed. The diameter of the larger drops was calculated by collecting the emitted droplets in a tared weighing bottle for a predetermined time interval, and

by subsequent weighings to measure the total mass. The number of drops actually collected was determined by measuring the frequency with which they were produced over a unit of time. From the weight and number of droplets and density of the original solution it was possible to calculate the droplet diameter. In the case of small droplets (below 120 microns diameter) the number of droplets produced over 2-3 minutes was too small to provide sufficient mass for accurate weighing. In this case the drop size was determined by collecting the droplets simultaneously on magnesium oxide coated glass slides and on the Printflex paper. The diameters of the craters produced by the drops impinging on the soft magnesium oxide layer were measured by means of a microscope (magnification 100 \times) using transmitted light. The crater diameter in microns multiplied by 0.85 equals the true drop diameter (6).

The diameters of the stains produced on the paper, beneath the glaze of the surface were measured with a Luminex magnifier equipped with an ocular micrometer (10 \times) for the larger stains, and by means of a microscope (100 \times) employing reflected light, for the measurement of the small stains. The Luminex, supplied by Beck (London), is a battery-operated, hand-held magnifier. The magnified field of vision is illuminated by an electric light bulb contained in the handle. In all cases the stains were measured after a period of 24 hours had elapsed, in order that the time-interval between sampling and measurement would be comparable to that which would elapse between field sampling of aerial sprays and laboratory assessment of the cards by the photographic method.

3. Results

The results of the calibration of spread factors for these two solutions on undyed Printflex paper are presented in Table 1. In the table, the

TABLE 1.—RELATION BETWEEN DROP DIAMETER AND STAIN DIAMETER ON PRINTFLEX PAPER FOR A SOLUTION OF 10% DDT IN AN OIL

Range of stain diameters (microns)	Mean diameter (microns)	Standard deviation	Drop diameter (microns) by "absolute" method	Drop diameter (microns) by MgO method	Standard deviation	Spread factor
<i>Determined by Luminex*</i>						
2750-3100	2950	108	446			6.6
2110-2470	2300	88	374			6.2
1220-1500	1350	58	243			5.6
1100-1300	1200	53	220			5.5
1040-1240	1150	51	202			5.7
910-1080	1000	46	183			5.5
880-1020	950	44	169			5.6
830-970	900	42	165			5.5
<i>Determined by Microscope**</i>						
465-535	500	26	108	111	5	4.6
395-485	440	24		101	5	4.4
365-455	410	22		95	5	4.3
330-410	370	20		88	4	4.2
300-380	340	19		81	4	4.2
275-335	305	18		75	4	4.1
190-250	220	14		59	3	3.7
<i>For a solution of 5% DDT in an oil</i>						
<i>Determined by Luminex*</i>						
2110-2490	2300	90	400			5.8
1670-2025	1850	75	325			5.7
1350-1650	1500	63	292			5.1
1040-1240	1150	51	203			5.7
830-970	900	42	176			5.1
400-500	450	24	110			4.1
290-360	325	19	80			4.1

* 100 stains measured in each case.

** 50 stains measured in each case.

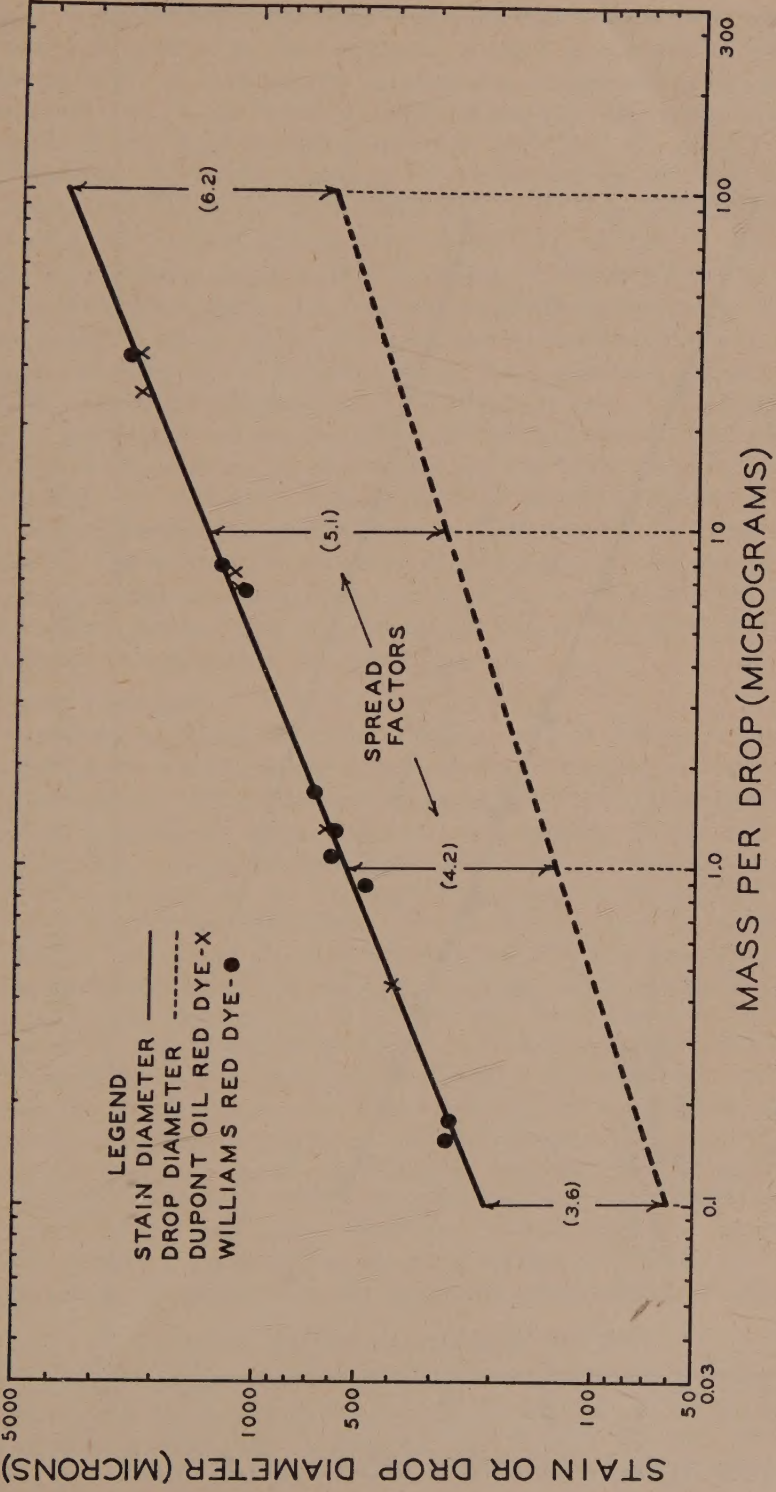


FIGURE 1. Spread of undyed 10% DDT solution on dyed Printflex paper.

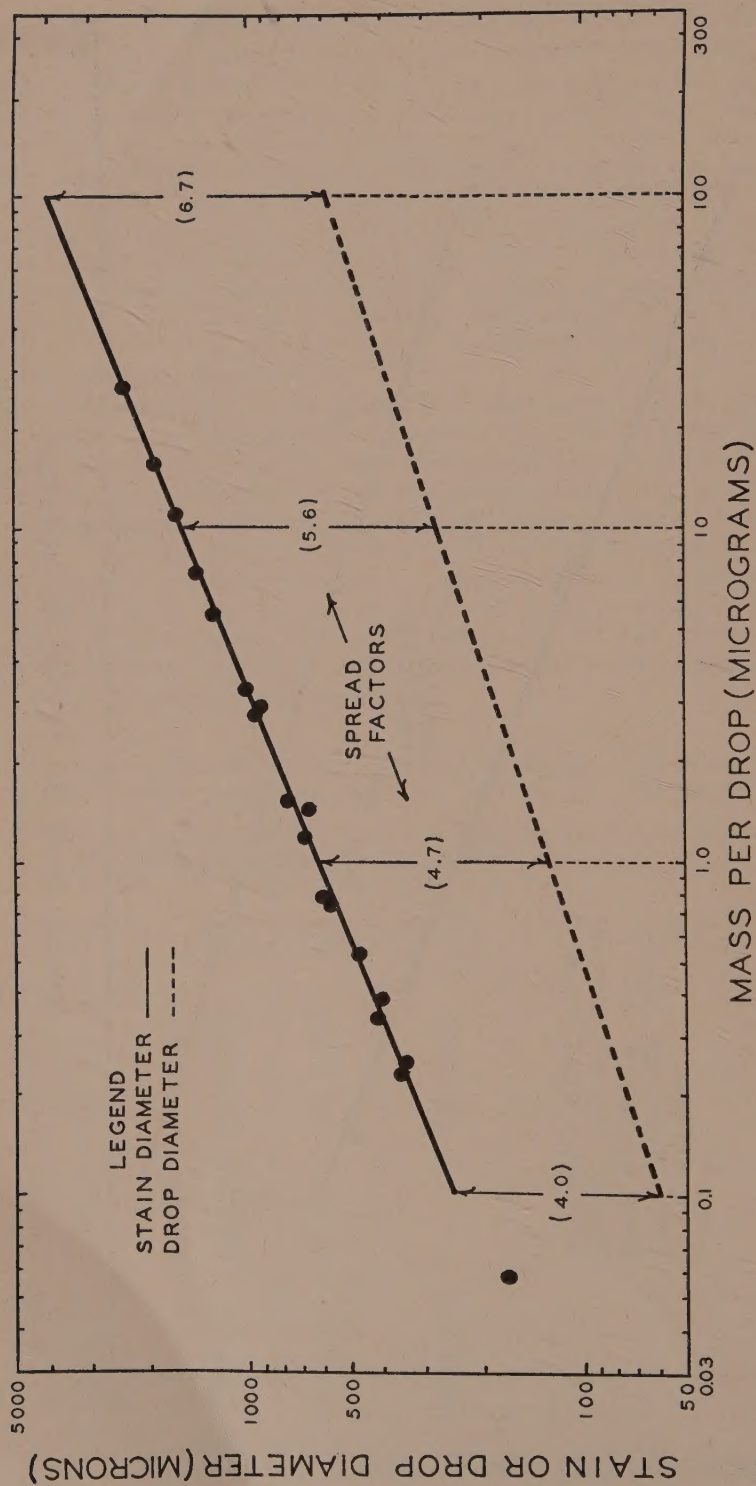


FIGURE 2. Spread of dyed 10% DDT solution on undyed Printflex paper.

method of measurement of drop diameter is indicated. The weighing method is referred to as the "absolute" method in contrast to the MgO crater method. It is of interest to note that, in the case of the droplets determined both by the "absolute" method and the MgO crater method, a difference of only three microns in diameter was noted, i.e. 108 microns for the "absolute" method as compared to 111 microns for the MgO method.

Davis and Elliott (1) have stated that, over a considerable range of drop sizes, the ratio of the diameter of the ring to that of the droplet producing it is fairly constant, from 1 : 6 to 1 : 7, depending on the characteristics of the spray used. Since experiments with undyed paper and dyed paper and dyed spray solution did not provide results comparable to those of these workers, dyed Printflex papers were prepared following their method, with both Williams' Red and du Pont Oil Red. Spread factors for a range of droplet diameters from 60 to 600 microns were determined. The variation in spread factor is not constant and the factor varies for this drop size range from 3.6 to 6.2. These data are illustrated in Figure 1. Similar data plotted for dyed spray droplets on undyed paper are illustrated in Figure 2. The spread factors are only slightly higher on the undyed paper, but these parallel the values found for dyed paper. The establishment of these spread factors may well allow the use of the dyed papers for quantitative assessments if the user so prefers.

Sampling Technique

For field sampling of aircraft sprays, a 2×3 inch rectangle of Printflex paper was stapled to a firm cardboard backing to ensure a flat sampling surface. This size was selected as being most convenient for the degree of enlargement sought and was approximately 50 per cent of the sampling surface presented by a Petri dish used for collecting samples for colorimetric analyses of dye content of the spray, i.e. 39 cm.² for Printflex card and 72 cm.² for Petri dish. One precaution must be observed in determining the size of the sampling surface. For the bulk of the spray swath, one sample of this size may be adequate. The number of droplets is normally large, ranging from 10 to 30 per cm.², and the mass per individual droplet small; and it is therefore possible to obtain a representative sample on this small size of sampling surface. However, it is frequently found that close to the aircraft track a variable small number of sampling positions will receive only a small number of very large drops, i.e. from less than one per cm.² to five per cm.², with the individual drop size 350 microns diameter and greater. Each drop at these sampling positions contributes from 5 to 25 per cent of the total mass. Therefore, in such cases it is necessary to increase greatly the size of the sampling surface to ensure that a large enough number of droplets are counted in the actual assessment procedure.

PHOTOGRAPHIC ASSESSMENT METHOD

Photographic Enlargement of Samples

The 2×3 inch sample card, properly identified, is placed in a recessed area of a removable easel constructed from $\frac{3}{4}$ inch plywood. A sponge

rubber pad holds the sample card smoothly and firmly against a 2 inch by 3 inch glass graticule. The glass graticule surface contains a ruled grid of 20 squares, each 1 centimetre square, and a series of numbered black circles corresponding to the range of stain sizes expected. The glass graticule can be eliminated if the same calibration material is printed on the surface of the cards. The sample card and the graticule are photographed simultaneously, using a bellows type 8×10 inch D2 Kodak copying camera, fitted with Kodak Commercial Ektar, f6.3, $8\frac{1}{2}$ inch lens supplemented with a Series VII 2 + Portra lens. This unit and the easel are rigidly bolted to a plywood frame mounted on a metal table. In the interval between recovery of sample cards in the field and photography, the samples are kept wrapped in light-tight paper, in order to maintain the greatest intensity of dyed stain. This greatly improves the contrast of the final image.

The easel is illuminated with two No. 1 G.E. Photoflood lamps, providing a total of 500 watts, mounted in permanently positioned reflectors on each side of the easel. A lens stop of f16 is used for a 5-second exposure which is controlled by a time-clock. This procedure provides consistent exposures.

Kodalith-Ortho Thin 8×10 inch paper is used instead of film. After processing in D85 developer for 2 minutes, followed by a stop bath of $2\frac{1}{2}$ per cent acetic acid for 10 seconds and fixing with F5 hypo for 6 minutes, the prints are washed for 20 minutes and dried.

The resulting finished print provides a white image on a black background, with the calibration graticule superimposed on the enlarged sample image. The enlargement obtained is approximately $3.5 \times$.

Assessment of Samples

For the actual assessment, the number of stains in each square is recorded first, and checked to ensure that the uniformity of the sample and total number of stains in the sample are adequate. The sizing process is carried out with a transparent plastic scale which is an exact replica of the enlarged scale of stain sizes on the original graticule. These two scales are checked against each other to guard against inadvertent small changes in the degree of enlargement. The true drop size is obtained by reference to prior calibrations carried out on Printflex paper, compared to the actual enlarged photographic stains. This is necessary to prevent errors due to distortion which may be caused by paper shrinkage. A sample card and the finished print of the same, ready for assessment, are shown in Figure 3.

This method allows the counting and sizing procedure to be carried out in an office or laboratory in which comfortable lighting and working conditions are available. In addition, no optical aids are required for the counting and sizing procedure. The several extra steps in processing that are required if film is used instead of paper are eliminated and a permanent record of the sample is provided for future reference.

ACCURACY OF THE ASSESSMENT METHOD

Procedure

1. Collection of Samples

The investigation of the accuracy of the photographic assessment method, as compared to the accuracy of other assessment methods in use at this

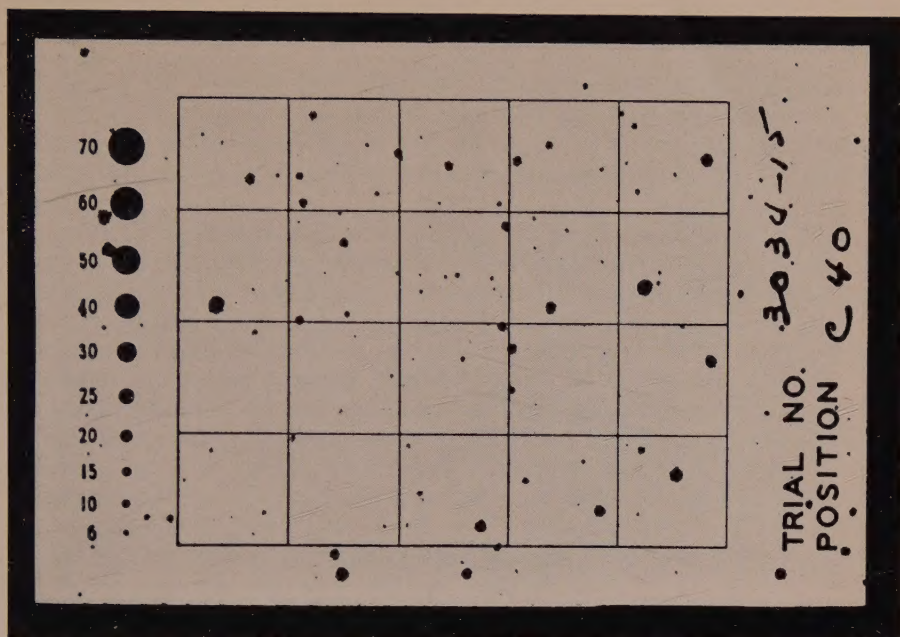


FIGURE 3a. Photograph of original 2 in. \times 3 in. sample card bearing stains of spray deposit.

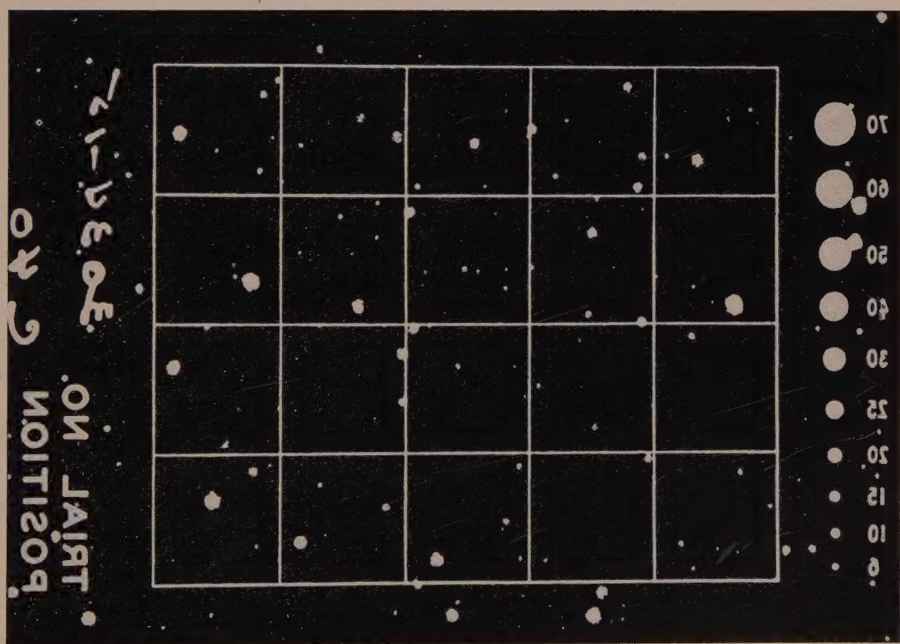


FIGURE 3b. Photograph of finished 8 in. \times 10 in. print ready for assessment.

laboratory, was carried out as part of the general investigation of spray sampling techniques. Two exploratory field trials were carried out to compare the various field sampling methods and methods of assessment. While it is necessary to consider some of the data on differences in the efficiency of field sampling in the statistical treatment of the assessment data, this report is concerned only with the accuracy of the various assessment methods.

Ten sampling positions, spaced at 20-yard intervals, were laid out in a line parallel to the wind direction. The spray solution was emitted from an aircraft flying at the height of 100 ft., on a track over the upwind end and at right-angles to the sampling line. At each sampling position two series of samples were taken, one on the ground and the other on a carefully levelled horizontal wooden platform 18 inches above the ground. Each sample consisted of five replicates of Petri dishes, measuring 9.6 cm. in diameter and with a 1 centimetre lip, Printflex cards 2×3 inches and glass slides 3×3.75 inches, i.e., equal in area to the Petri dishes, all on the platform, and 2×3 inch glass microscope slides on the ground. All sets of samples were grouped on an 18×18 inch jump card. All glass surfaces were treated with G.E. Dri-film 9987 in order to facilitate deposit removal, and to retain the deposit in discrete droplet form.

Two trials were carried out, the first using a spray solution containing 5 per cent DDT, and the second, a spray containing 10 per cent DDT. The DDT was dissolved in a Velsicol AR-50 fuel oil mixture and dyed with 0.5 per cent Williams' Red.

2. Assessment Methods

(i) Colorimetric

A sample of the dyed spray solution was taken from the aircraft spray tanks while they were being filled, and a second sample was taken from the tanks after each trial. A standard curve was prepared from colorimetric analyses of solutions containing known dye concentrations in the solvent mixture used for extraction from glass surfaces (xylene: kerosene, 1:3 v/v). An Evelyn photoelectric colorimeter with a No. 515 filter was used for these analyses. Deposits on Petri dishes were extracted with 10 ml. of solvent and transferred to colorimeter tubes for determination. The deposits on glass slides were extracted by placing each slide in a glass funnel and applying 10 ml. of solvent as a fine stream to the slide. Usually all traces of colour were removed after the application of the first 2 to 3 ml. of solvent. The total weight or volume of spray solution recovered from each sample of the deposit was calculated as mgm. per cm.² or gallons per acre.

(ii) Printflex Paper Samples

The samples collected on Printflex paper were assessed for number of drops per square centimetre, volume of deposit in terms of gallons of spray solution per acre, mass median diameter and number median diameter. The droplet analyses were carried out by three different methods, with the same personnel carrying out all analyses. The methods employed were:

(a) Vickers Projection Microscope

The Vickers projection microscope is an inverted type projection microscope. That is to say, the specimen is positioned above the objective. The microscope is manufactured and supplied by Cooke, Troughton and Simms, York, England. With this method, the enlarged image of the sample card, along with a calibrated reference scale composed of a series of calibrated circles of graded size, was projected on to the translucent 4 × 6 inch glass screen of the microscope. The most suitable magnification for this purpose, with this instrument, was 10 ×, obtained by employing the $3\frac{1}{4}$ inch f4.5 photographic lens and suitable bellows extension. This resulted in a field of only 1 centimetre square being counted at one time. Since this method employed the smallest size field with greatest resolution of image, it was considered to be the most accurate in selecting a reference standard. The actual sizing was carried out using a calibrated transparent plastic scale, so that each stain was actually fitted into its proper size grouping by measurement rather than by eye. The graticule scale was projected along with the sample in order to ensure constant enlargement from sample to sample. While this method was the most accurate, it was found to be the most tiresome and time-consuming of the three.

(b) Epidiascope

An Aldis epidiascope with 35 cm., f4 lens, manufactured by Aldis Bros., Birmingham, England, was employed to project the image of 20 square centimetres of the sample card surface on to a 20 × 18 inch translucent screen. The resolution by this method was not so good as that obtained with the other methods. Stains were individually sized by the same method used in the Vickers projection and photographic methods. The large size and brightness of the screen reduced the efficiency of this method. This was due to the discomfort suffered by the operators in the counting and sizing procedure.

(c) Photographic Method.

3. Results and Discussion

(i) Statistical Analysis

From the data on droplet size and number per cm.², it was possible to calculate the mass recovered per cm.² by the three above methods, for comparison with mass recovery data obtained from colorimetric analyses.

The data from the colorimetric analyses and droplet assessments for the two trials were calculated independently or pooled if required, for the purposes of statistical examination. While space does not justify the inclusion of all the data that were used in arriving at the results recorded here, a few samples of data obtained from the centre of the sampling line are presented in Table 2. This table illustrates the inherent variations in levels of deposit encountered at these sampling positions as determined by the different methods of assessment.

The statistical examination and comparison of the pooled data were carried out by employing the statistics of the straight line as outlined by Youden (8). When pairs of data were being compared, a plot of one set of data against the other would result in a straight line of slope not significantly different from one, provided no difference could be proven between the methods of assessment. Student's "t" test at the 5 per cent probability level was employed to determine the significance of the difference which might occur.

(ii) Mass of Deposit

A comparison was made of mass of deposit data obtained from glass slides and similar data obtained from the Printflex cards, with the cards assessed by the three different methods. These comparisons reveal that (a) no significant difference was proven between ground deposits calculated by both the Vickers Projection Microscope, and photographic assessments of the Printflex cards, and deposits determined by colorimetric assessment of the dye tracer collected on the glass slides at the 95 per cent confidence limits; and, (b), ground deposits calculated from the Epidiascope assessments of Printflex cards were significantly lower than deposits on glass slides by $13.0\% \pm 0.7$ per cent at the 95 per cent confidence limits.

A comparison of deposits on Petri dishes and deposits on glass slides was carried out in order to determine whether or not the shading effect of the lip of the Petri dish would have any effect on the sampling efficiency of this unit. The flat glass slide surfaces were considered to be equivalent to the Printflex card surfaces in efficiency of sampling the depositing spray. Comparison of data from the two trials showed that the slopes of the lines of plotted data were not proven to differ significantly from one another but did differ significantly from one. By pooling the data, calculating a new average slope and applying the "t" test, a difference in sampling efficiencies was firmly indicated. At the 95 per cent confidence limits, the deposits on Petri dishes were on the average, $10.2\% \pm 0.8$ per cent higher than on the glass slides. This indicates that any reduction of deposit by the shading effect of the upwind exterior lip, which was wiped clean before analysis of the interior dish contents, is overshadowed in importance by the higher efficiency of droplet collection which is characteristic of the interior downwind lip surface.

The calculated deposit data obtained from all sampling points, as determined by the Vickers projection microscope, Epidiascope and Photographic methods, were pooled for each method. Analysis of these data revealed that no difference could be established between the Vickers projection microscope and photographic methods. The Epidiascope assessment technique was shown to produce calculated deposit data which on the average was significantly less than similar data from the Vickers technique by $8.7\% \pm 0.2$ per cent at the 95 per cent confidence limits.

(iii) Density of Deposit

The droplet density data, in terms of drops per square centimetre, for the two trials, were pooled for each assessment method. The analyses of these data revealed that the intercepts, which represent constant errors, were significantly different from zero. However, in each case, the values of the constant errors were less than one drop per square centimetre, which is not considered to be significant from the standpoint of the application of the results obtained from this type of experiment. Disregarding the constant errors and recalculating the slopes demonstrated that:

- (a) No significant difference was proven between the droplet density counts obtained by means of the Vickers projection microscope and Epdiascope assessment methods.
- (b) The photographic assessment method produced droplet density data that were higher than the similar data produced by the Vickers projection microscope method by $1.2\% \pm 0.1$ per cent at the 95 per cent confidence limits.

Therefore, for all practical experimental applications, the three methods of assessing droplet density can be considered to be equivalent and accurate.

(iv) Droplet Size

The mass median diameter data obtained by the three methods assessment were treated in the same fashion as the other parameters of the deposit. No significant differences were proven between the Vickers projection microscope and Epdiascope methods. Mass median diameters obtained by the photographic droplet assessment method were found to be $1.8\% \pm 0.1$ per cent higher than those obtained by the Vickers projection microscope method at the 95 per cent confidence limits. For all practical applications in the experimental work, this difference is not considered to be significant.

CONCLUSIONS

For accurate experimental work it is necessary to establish spread factors for stains on Printflex paper for each experimental solution. With dyed droplets of DDT-solution applied to undyed Printflex paper, the spread factor has been determined to vary between 3.6 to 6.2 for stains produced by droplets ranging from approximately 60 to 600 microns in diameter. The spread factors for undyed solutions on pre-dyed paper, are similar in magnitude to those for undyed paper, i.e. they are not constant, and cover a wide range corresponding to the true drop sizes.

The photographic assessment method has been found to be comparable in accuracy to the other conventional methods of assessment for determining the volume of deposit, and for determining the various droplet parameters required for experimental work requiring a high level of accuracy.

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CHEMICAL COMPOSITION OF NINE GRASSES AT SIX STAGES OF DEVELOPMENT¹

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ABSTRACT

Nine grasses (smooth brome grass, Russian wild ryegrass, Mandan wild ryegrass, Fairway crested wheatgrass, Summit crested wheatgrass, intermediate wheatgrass, tall wheatgrass, streambank wheatgrass, and Green Stipagrass) grown in a field test of the random block split-plot design were harvested at six stages of development (early leaf, shot blade, start of flowering, mature seed, late fall, and early next spring). The cuttings were analysed for proximate constituents, lignin, calcium, and phosphorus and the data were analysed statistically.

There were significant differences in protein, fat, crude fibre, ash, lignin, and nitrogen-free extract between species and between stages of growth. Streambank wheatgrass retained the highest fat content after winter exposure but Russian wild ryegrass and Green Stipagrass also contained relatively more fat than other species. The same three grasses were lowest in lignin at the cured stage and had more favourable calcium to phosphorus ratios at that stage than all grasses except tall wheatgrass. Russian wild ryegrass was highest in protein at the cured stage and streambank wheatgrass was second highest.

The chemical data suggest the use of crested wheatgrass or intermediate wheatgrass for spring and early summer grazing, brome grass or Green Stipagrass for summer grazing, and Russian wild ryegrass and streambank wheatgrass for fall and possibly winter grazing.

INTRODUCTION

It has been well established that the nutritive value of grasses varies from one stage of development to another within a species and also between species at particular stages (3, 4, 5, 6, 7, 9, 15, 16, 17 and 20). Although the nutritive value as determined by chemical analysis is not an absolute measure of the feed value of a crop, it is generally a good indication of its value (1, 2, 7, 11). Palatability, of course, enters into the picture and, in some instances, may be the determining factor in the value of a feed (5, 19).

The present study was undertaken with the object of determining, by chemical analysis, the relative nutritive value of nine cultivated grasses at various stages during the growing season and after curing. A grass is considered to cure well when it retains a relatively high nutritive value after maturity and at the same time preserves its physical form many months after growth ceases. Pigden (18) described this quality and studied relative curing qualities of several cultivated and native grasses. He concluded that crested wheatgrass and brome grass, the most commonly used cultivated grasses in the Canadian Prairies, had inferior curing qualities compared to several native grasses studied.

A high nutritive value in a grass after curing is desirable in the prairies of Western Canada because considerable grazing occurs in late summer and in fall after growth has ceased.

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Although general experience with cultivated grasses substantiates Pigden's findings, the quality of rangeland pastures seeded to cultivated grasses would be improved if a cultivated grass could be found that is superior to brome grass and crested wheat grass in curing quality. In addition, a thorough knowledge of the relative nutritive value would help the pasture manager to decide how he might use various grasses in a pasture rotation in a semi-arid climate to the best advantage. In this study the various chemical constituents of a number of "new grasses" are compared at different times of the year with those of brome grass and crested wheat grass.

MATERIALS AND METHODS

The grasses studied were:

Smooth brome grass	— <i>Bromus inermis</i> Leyss.
Russian wild ryegrass	— <i>Elymus junceus</i> Fisch.
Mandan wild ryegrass	— <i>Elymus canadensis</i> L.
Fairway crested wheat grass	— <i>Agropyron cristatum</i> (L.) Gaertn.
Summit crested wheat grass	— <i>Agropyron desertorum</i> (Fisch.) Beauv.
Intermediate wheat grass	— <i>Agropyron intermedium</i> (Host.) Beauv.
Tall wheat grass	— <i>Agropyron elongatum</i> (Host.) Beauv.
Streambank wheat grass	— <i>Agropyron riparium</i> Scribn. and Smith
Green Stipagrass	— <i>Stipa viridula</i> Trin.

The grasses were seeded on May 3, 1950, at Swift Current, Saskatchewan, on loam soil of good natural fertility. The test was of the random block design. The species constituted the main plots and each main plot consisted of six sub-plots of a row each to provide material for the determination of nutritive value at six different stages during the year. The sub-plots were randomized within each main plot, making the design a split-plot type. A main plot consisted of six rows, 20 feet long, and spaced 18 inches apart.

Cuttings for chemical analyses were taken at the following stages of development in 1951 and 1952: Early leaf; shot blade; start of flowering; mature seed; cured (cut late in fall), and cured (cut next spring after overwintering). After cutting, the samples for all analyses were oven-dried at 190° F., except samples for lignin analyses which were air-dried. The dried samples were pulverized in an 8-inch laboratory hammermill with 20 mesh screen and sub-samples were taken from the pulverized material for analysis.

Standard procedures (13) were used for determining crude protein, fat, crude fibre, and ash, except that fat was extracted for 4 hours in the Goldfisch apparatus with petroleum ether. Nitrogen-free extract was calculated by subtracting from 100 gm. dry matter the sum of crude protein, fat, crude fibre, and ash. Lignin was determined by the 72 per cent sulphuric acid method (8). The method for phosphorus was adapted from that of King (10) and calcium was determined by McCance's procedure (12).

Variance analyses were made of the 2-year data separately for every stage for each feed constituent. The differences between stages were generally so great that no statistical analysis was necessary to substantiate their validity.

The weather was quite favourable to growth during both 1951 and 1952 but the fall in 1952 was drier than in 1951 and there was little grass growth after August 15 that year.

TABLE 1.—PROTEIN CONTENT OF NINE GRASSES AT SIX STAGES OF DEVELOPMENT ON DRYLAND
Two-Year Results, 1951 and 1952

Species	Protein Per Cent at Stages					
	Early leaf	Shot blade	Flower	Mature seed	Late fall	Next spring
Bromegrass	20.7	17.1	10.4	7.3	4.5	4.6
Russian wild ryegrass	21.1	16.4	11.5	9.4	7.3	7.1
Mandan wild ryegrass	22.0	13.5	6.7	5.0	2.5	2.1
Fairway crested wheatgrass	20.1	14.2	6.4	5.4	3.1	3.4
Summit crested wheatgrass	20.4	14.8	6.6	6.0	3.8	3.3
Intermediate wheatgrass	21.3	11.7	6.6	4.2	3.2	3.2
Tall wheatgrass	18.9	12.1	6.3	4.6	3.2	3.1
Streambank wheatgrass	17.4	13.5	9.2	7.1	5.5	5.9
Green Stipagrass	17.6	12.6	8.7	6.2	4.0	4.2
Mean for stages—2 years	19.9	14.0	8.0	6.1	4.1	4.1
L.S.D. (P = .05)	1.5	1.6	1.0	1.0	0.7	0.4
Mean for 1951	25.1	16.6	9.7	7.0	5.1	5.4
Mean for 1952	14.8	11.2	6.3	5.3	3.1	2.8
L.S.D. Years (P = .05)	0.8	0.5	0.6	1.0	0.4	0.7

TABLE 2.—NITROGEN-FREE EXTRACT CONTENT OF NINE GRASSES AT SIX STAGES OF DEVELOPMENT ON DRYLAND
Two-Year Results, 1951 and 1952

Species	Nitrogen-Free Extract Per Cent at Stages					
	Early leaf	Shot blade	Flower	Mature seed	Late fall	Next spring
Bromegrass	43.9	46.5	53.1	54.3	49.9	48.3
Russian wild ryegrass	42.9	46.6	46.3	44.9	45.7	44.3
Mandan wild ryegrass	41.7	49.3	51.8	53.8	46.9	46.6
Fairway crested wheatgrass	44.7	49.8	55.0	55.7	49.7	47.9
Summit crested wheatgrass	43.8	49.0	54.6	55.5	48.7	47.4
Intermediate wheatgrass	40.9	50.5	54.1	54.0	48.5	47.2
Tall wheatgrass	41.5	47.2	48.8	49.0	48.3	47.4
Streambank wheatgrass	41.7	46.9	47.3	46.5	45.4	43.6
Green Stipagrass	46.5	48.9	50.1	49.3	49.1	48.1
Mean for stages—2 years	43.0	48.3	51.2	51.4	48.0	46.8
L.S.D. ($P = .05$)	1.4	0.8	1.6	1.3	1.2	1.2
Mean for 1951	38.8	46.7	49.8	51.0	46.7	45.7
Mean for 1952	47.4	49.9	52.7	51.9	49.3	47.9
L.S.D. Years ($P = .05$)	1.0	1.0	0.8	0.8	0.4	0.5

TABLE 3.—ETHER EXTRACT CONTENT OF NINE GRASSES AT SIX STAGES OF DEVELOPMENT ON DRYLAND
Two-Year Results, 1951 and 1952

Species	Ether Extract Per Cent at Stages					
	Early leaf	Shot blade	Flower	Mature seed	Late fall	Next spring
Bromegrass	2.28	2.01	1.47	1.84	1.36	0.95
Russian wild ryegrass	1.71	1.56	1.40	1.71	1.62	1.16
Mandan wild ryegrass	2.27	1.54	1.15	1.32	1.25	0.67
Fairway crested wheatgrass	2.18	1.71	1.42	1.74	1.12	0.65
Summit crested wheatgrass	2.04	1.62	1.19	1.55	1.35	0.79
Intermediate wheatgrass	2.40	1.81	1.78	1.88	1.33	0.77
Tall wheatgrass	2.09	1.78	1.69	1.55	1.26	0.52
Streambank wheatgrass	2.40	2.31	1.89	2.63	2.08	1.76
Green Stipagrass	1.64	1.53	1.10	1.28	1.43	1.07
Mean for stages—2 years	2.11	1.76	1.45	1.72	1.42	0.92
L.S.D. (P = .05)	0.14	0.09	0.14	0.29	0.17	0.17
Mean for 1951	2.52	1.99	1.61	1.81	1.60	0.96
Mean for 1952	1.81	1.53	1.29	1.63	1.24	0.88
L.S.D. Years (P = .05)	0.11	0.03	0.11	0.11	0.14	0.11

TABLE 4.—CRUDE FIBRE CONTENT OF NINE GRASSES AT SIX STAGES OF DEVELOPMENT ON DRYLAND
Two-Year Results, 1951 and 1952

Species	Crude Fibre Per Cent at Stages					
	Early leaf	Shot blade	Flower	Mature seed	Late fall	Next spring
Bromegrass	22.4	24.4	28.2	28.5	36.4	37.0
Russian wild ryegrass	24.0	26.5	32.8	34.7	37.9	38.8
Mandan wild ryegrass	23.7	27.6	34.0	34.2	43.7	45.4
Fairway crested wheatgrass	24.0	26.4	31.1	30.6	38.8	40.3
Summit crested wheatgrass	25.0	26.9	32.3	31.4	40.4	42.9
Intermediate wheatgrass	23.1	28.0	30.9	33.2	39.3	40.7
Tall wheatgrass	25.4	30.4	36.6	38.1	41.1	42.9
Streambank wheatgrass	28.2	29.1	34.7	35.1	39.2	38.6
Green Stipagrass	24.7	29.6	34.1	36.7	39.6	40.0
Mean for stages—2 years	24.5	27.6	32.7	33.6	39.6	40.7
L.S.D. ($P = .05$)	0.9	1.4	1.3	1.4	1.5	1.5
Mean for 1951	22.6	26.1	32.5	33.8	39.9	40.8
Mean for 1952	26.5	29.2	33.0	33.4	39.2	40.7
L.S.D. Years ($P = .05$)	0.6	0.9	N.S.	N.S.	N.S.	N.S.

EXPERIMENTAL RESULTS

Protein (Table 1)

The protein content was lower in 1952 than in 1951 at all stages for all species. The 2-year analysis shows that there were highly significant differences in protein content between species at all stages. Russian wild ryegrass and streambank wheatgrass were outstanding, in that they retained a high protein content late into the season after "curing". Russian wild ryegrass was one of the top grasses in protein content at all stages. There was a significant interaction between species and years in protein content at several stages, possibly because of different degrees of heading of species in the two years.

Nitrogen-Free Extract (Table 2)

The nitrogen-free extract was higher in 1952 than in 1951 for all species at all stages. In general, the species high in protein were correspondingly lower in nitrogen-free extract content; brome grass appeared to be somewhat of an exception in this regard, being relatively high in both protein and nitrogen-free extract. Russian wild ryegrass and streambank wheatgrass were significantly lower in nitrogen-free extract content than all other species at the flowering stage and later stages.

Fat (Ether Extract) (Table 3)

The fat content of the forage was substantially higher in 1951 than in 1952. The 2-year analysis shows that there were significant differences in fat content between species at every stage. Streambank wheatgrass contained significantly more fat than practically all species at every stage. It was outstanding in this respect late in the fall and after overwintering in the field. This grass may be useful in the range area for cold weather grazing, since its calorific value appears to be higher than that of the other grasses.

Crude Fibre (Table 4)

At early stages of growth, crude fibre content was significantly higher in 1952 than in 1951, but at the flowering stage and later there was no significant difference between years. There was, however, a significant interaction between species and years for fibre content at the various stages. This could be due to different degrees of culm formation in the 2 years. The analysis shows that brome grass was low in fibre at all stages, while Mandan wild ryegrass and tall wheatgrass contained a high amount of fibre at all stages after the flowering stage. The low fibre content of brome grass could be the reason for its generally accepted high palatability. Russian wild ryegrass was also relatively low in fibre content late in the fall and after overwintering in the field.

Lignin (Table 5)

Analyses for lignin content were made only in 1951. There appears to be a fairly close general agreement between amount of crude fibre and lignin in the species tested. It appears that the species in which leaves are located mostly at the base and which produce culms more sparsely contain

TABLE 5.—LIGNIN CONTENT OF NINE GRASSES AT FOUR STAGES OF DEVELOPMENT ON DRYLAND

Species	1951 Results			
	Lignin per cent at stages			
	Early leaf	Flower	Mature seed	Cured—late fall
Bromegrass	4.6	9.2	11.2	14.6
Russian wild ryegrass	5.4	9.4	10.7	12.2
Mandan wild ryegrass	5.0	11.4	12.9	17.0
Fairway crested wheatgrass	5.4	11.5	13.6	15.0
Summit crested wheatgrass	6.0	11.4	11.9	15.7
Intermediate wheatgrass	4.3	9.8	11.7	15.0
Tall wheatgrass	5.5	11.8	12.7	13.6
Streambank wheatgrass	6.8	10.4	10.9	13.1
Green Stipagrass	6.4	9.2	11.6	12.9
Stage Mean	5.5	10.5	11.9	14.3
L.S.D. (P = .05)	0.8	1.2	N.S.	1.6

less lignin after maturity than the species which form culms freely. For example, Russian wild ryegrass and Green Stipagrass in which leafage is basal, are relatively low in lignin content.

Ash (Table 6)

The ash content in 1952 was lower than in 1951 during the early stages of development and higher after maturity. This probably was the case because 1952 was dry after midsummer and leaves dried up, while 1951 was wet through August and active growth continued until much later in the season.

Phosphorus and Calcium (Table 7)

These elements were determined in 1952 only. A calcium-to-phosphorus ratio ranging between 1 : 1 and 2 : 1 is highly desirable. However, where there is an ample supply of vitamin D, as in sun-cured forages, a satisfactory ratio may be much greater than this (14). There was a good balance of calcium to phosphorus in all grasses up to the mature seed stage, but late in the fall and next spring certain grasses were superior to others. The following ratios were found late in the fall:

Bromegrass	12 : 1
Russian wild ryegrass	5 : 1
Mandan wild ryegrass	8 : 1
Fairway crested wheatgrass	7 : 1
Summit crested wheatgrass	7 : 1
Intermediate wheatgrass	7 : 1
Tall wheatgrass	5 : 1
Streambank wheatgrass	5 : 1
Green Stipagrass	6 : 1

Russian wild ryegrass, tall wheatgrass, streambank wheatgrass and Green Stipagrass had the most favourable ratios at this stage.

TABLE 6.—ASH CONTENT OF NINE GRASSES AT SIX STAGES OF DEVELOPMENT ON DRYLAND
Two-Year Results, 1951 and 1952

Species	Ash Per Cent at Stages					
	Early leaf	Shot blade	Flower	Mature seed	Late fall	Next spring
Bromegrass	10.6	10.0	6.8	8.0	7.8	9.3
Russian wild ryegrass	10.2	8.9	8.0	9.2	7.5	8.6
Mandan wild ryegrass	10.3	8.1	6.4	5.7	5.7	5.2
Fairway crested wheatgrass	9.0	7.8	6.0	6.7	7.3	7.8
Summit crested wheatgrass	8.8	7.6	5.3	5.6	5.8	5.6
Intermediate wheatgrass	12.2	8.0	6.6	6.7	7.7	8.0
Tall wheatgrass	12.0	8.5	6.6	6.7	6.2	6.1
Streambank wheatgrass	10.3	8.3	6.9	8.6	7.9	10.2
Green Stipagrass	9.6	7.8	6.2	6.6	5.9	6.7
Mean for stages—2 years	10.3	8.3	6.5	7.1	6.9	7.5
L.S.D. (P = .05)	0.5	0.7	0.8	0.9	1.1	1.9
Mean for 1951	11.0	8.6	6.3	6.4	6.6	7.2
Mean for 1952	9.7	8.1	6.7	7.8	7.1	7.8
L.S.D. Years (P = .05)	0.8	0.5	N.S.	0.5	N.S.	N.S.

TABLE 7.—CALCIUM AND PHOSPHORUS CONTENT OF NINE GRASSES AT SIX STAGES OF DEVELOPMENT ON DRYLAND—1952 RESULTS

Species	Early leaf		Shot blade		Flower		Mature seed		Late fall		Next spring	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Bromegrass	.25	.23	.24	.21	.25	.12	.34	.09	.37	.03	.39	.03
Russian wild ryegrass	.21	.22	.21	.21	.19	.13	.26	.10	.30	.06	.27	.03
Mandan wild ryegrass	.26	.25	.29	.17	.18	.10	.19	.13	.17	.02	.17	.02
Fairway crested wheatgrass	.20	.19	.22	.17	.23	.10	.28	.07	.27	.04	.23	.03
Summit crested wheatgrass	.21	.21	.24	.17	.19	.11	.25	.09	.23	.03	.22	.03
Intermediate wheatgrass	.21	.21	.22	.13	.24	.07	.28	.05	.22	.03	.20	.03
Tall wheatgrass	.21	.21	.25	.16	.19	.10	.20	.07	.20	.04	.16	.04
Streambank wheatgrass	.26	.18	.26	.16	.25	.13	.33	.10	.32	.06	.31	.06
Green Stipagrass	.21	.18	.20	.15	.14	.12	.15	.08	.19	.03	.19	.03
Mean	.22	.21	.24	.17	.21	.11	.25	.09	.25	.04	.24	.03

DISCUSSION

Russian wild ryegrass, streambank wheatgrass and Green Stipagrass were lowest in lignin content at the mature stage and retained a relatively high fat content at the cured stage. In this respect they were similar to two native grasses, *Stipa comata* Trin. & Rupr. and *Festuca scabrella* Torr., which were described by Pigden (18) and which are accepted as being of relatively high nutritive value in the cured state. In addition, Russian wild ryegrass and streambank wheatgrass had a relatively high protein content in the cured stage, the former being outstanding in this respect.

Russian wild ryegrass appears to be the most nutritive species in the test. The data indicate that there is less danger of losing nutritive value by delayed utilization in Russian wild ryegrass than in the other species. Its apparently excellent curing qualities should make it a useful grass for grazing during late summer and fall, and perhaps during winter in regions of low rainfall. Streambank wheatgrass and Green Stipagrass also appear to have qualities which may make them useful for late summer and fall grazing, being relatively better than crested wheatgrass and intermediate wheatgrass after mid-summer. The relatively high fat content of streambank wheatgrass at the cured stage suggests that it may be a useful pasture grass for cold weather grazing by range cattle.

The chemical data suggest the use of crested wheatgrass and intermediate wheatgrass for spring and early summer grazing, brome grass and Green Stipagrass for summer grazing, and Russian wild ryegrass and streambank wheatgrass for fall and possible winter grazing. Actual grazing tests have been initiated by the Experimental Farm, Swift Current, Saskatchewan, in order to obtain further information on different grasses for rotational grazing in the dry prairies.

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CROSS COMPATIBILITY RELATIONSHIPS AMONG SOME *AVENA* SPECIES AND POLYPLOIDS¹

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ABSTRACT

Compatibility relationships were studied in crosses among 8 annual *Avena* species representing the diploid, tetraploid, and hexaploid groups. The relative self fertility of hybrids and parental species was obtained. Crosses which failed to produce seed, and interspecific hybrids which were sterile on selfing, were studied to determine the most critical stages at which failures of the reproductive processes occurred. Colchicine treatments were used to induce chromosome doubling of some of the low chromosome species and the highly sterile hybrids. The following artificial polyploids were produced: Autotetraploids of *A. strigosa* and *A. brevis* ($2n = 28$); amphiploids of *A. abyssinica* \times *A. sterilis* ($2n = 70$); *A. abyssinica* \times *A. sativa* ($2n = 70$); and *A. abyssinica* \times *A. strigosa* ($2n = 42$). Crosses were made between the autotetraploid of *A. strigosa* and species of each of the chromosome groups and seed set was compared with crosses involving the diploid *A. strigosa*. Crosses were made between the artificially induced polyploids and *A. sativa* in an effort to transfer genes from the lower chromosome species to common oats.

INTRODUCTION

Some of the less common species of *Avena*, particularly those in the diploid group, possess desirable agronomic characteristics such as good kernel quality, resistance to various diseases such as crown and stem rust, loose and covered smut, powdery mildew, and black stem. If successful gene transfer could be accomplished between the diploid or tetraploid species and the hexaploid species, additional sources of desirable germ plasm would be available in a practical oat improvement program. No desirable agronomic characters are known to the author to have been transferred from species of the lower chromosome groups to the hexaploid species. Incompatibility between two species is considered to exist if hybrid plants cannot be obtained by cross-pollination, whereas the inability of the hybrids to produce viable seed is referred to as sterility. Both phenomena occur to a greater or lesser extent in hybridization among oat species of different chromosome groups, and are an important impediment to gene transfer.

The present study was carried out at Iowa State College, Ames, Iowa, and the Central Experimental Farm, Ottawa, during 1953 and 1954. The aspects considered were: Cross compatibility relationships among the annual species of *Avena*, and the relative self fertility of the hybrids obtained; critical stages at which reproductive failures tend to occur following cross-pollination; measures which may be used to overcome the barriers to compatibility and fertility; compatibility between artificially produced polyploids and the cultivated species.

Extensive research on the cytogenetic relationships among *Avena* species was undertaken by Nishiyama in 1929 and is still continuing. His work has been reported in a series of publications (8, 11, 12, 13, 14). Sampson (16) has recently reviewed much of the literature dealing with cytological and genetic relationships in oats. Interspecific hybrids and

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²Cereal list.

amphiploids have been obtained by Nishiyama (11, 14), Shepelva (17), Fetissoff (6), Lesik (10), Cameron and Garvin (3, 4), Griffiths*, and Brown and Shands (2).

MATERIALS AND METHODS

Cross pollinations were made among varieties belonging to the following annual *Avena* species: *A. brevis* Roth., *A. wiestii* Steudel, *A. strigosa* Schreb. and *A. nudi-brevis* Vav. of the diploid group ($2n = 14$); *A. barbata* Pott and *A. abyssinica* Hochst. of the tetraploid group ($2n = 28$); and *A. byzantina* Koch., *A. sativa* L., *A. fatua* L., and *A. sterilis* L. of the hexaploid group ($2n = 42$). The crosses were made in the greenhouse at Ames during the 1952-53 and 1953-54 seasons and in the nursery at Ottawa during the summer of 1953. Bagging was necessary in the nursery to prevent outcrossing but not under greenhouse conditions.

To obtain a measure of the relative self-fertility of the hybrids obtained from the crosses, they were grown along with the parental varieties in the greenhouse at Ames during the winter of 1953-54. The greenhouse was preferred because of the relative freedom from outcrossing. The average number of seeds set per spikelet was used as a measure of self-fertility.

Meiotic behaviour of the hybrids was observed on pollen mother cell preparations stained with aceto-carmin after fixing in modified Carnoy's solution. Hanson and Oldemeyer's (7) aceto-carmin smear technique provided a rapid method for root tip counts.

Microscopic observations were made at various stages from germination of pollen to seed formation to determine the extent of reproductive development following cross pollination. Ovary stimulation was observed among crosses which produced no viable seed. One such incompatible cross (*A. strigosa* \times *A. sativa*) was chosen for a more intensive study. Ovaries were removed at intervals of 2, 4, 8, and 16 days after pollination. These were embedded in wax, sectioned, and the relative development of the hybrid ovaries was compared with that of the parental varieties.

One of the most effective means of overcoming the sterility frequently occurring in species hybrids is by artificially inducing chromosome doubling. Several methods of treatment using aqueous colchicine and one using acenaphthene, as described by Kostoff (9), and Cameron and Garvin (3), were tested on the species *A. strigosa* var. C.D. 3820. The most effective method was then used to double the chromosome number of the sterile hybrids. Observations were made on stomata and pollen size, and on the fertility of the treated plants to determine the effect of the treatment. Seeds from plants suspected of having the doubled chromosome number were germinated and chromosome counts were made on their root tips to establish whether doubling had actually occurred.

RESULTS AND DISCUSSION

Species Crosses

The results of crosses made are summarized in Table 1. As the species used within each of the chromosome groups were highly compatible with one another the crosses are listed by groups rather than by species. The seed set in crosses between species having the same chromosome number

*Griffiths, D. J. Personal communication. Welsh Plant Breeding Station, Aberystwyth, Wales. 1954.

TABLE 1.—SUMMARIZED RESULTS OF THE CROSSES MADE AMONG *Avena* SPECIES

Species involved ¹	No. of florets pollinated	No. of hybrid seeds obtained	Per cent seed set	No. of seeds germinated
Hexaploid × hexaploid	728	356	48.9	347
Hexaploid × tetraploid	341	10	2.9	2
Hexaploid × diploid	351	0	0	0
Tetraploid × hexaploid	522	129	24.7	106
Tetraploid × tetraploid	53	9	16.9	9
Tetraploid × diploid	246	31	12.6	30
Diploid × hexaploid	291	0	0	0
Diploid × tetraploid	66	8	12.1	0
Diploid × diploid	109	46	42.2	41

¹The female parent species is listed first in recording the cross.

was relatively high. The percentage of successful crosses obtained between tetraploid × hexaploid species and between tetraploid × diploid species was generally lower than crosses within groups, but successful crosses were still readily obtained. The following species crosses either resulted in a very low seed set or failed to produce normal seeds: Diploid × tetraploid; diploid × hexaploid; hexaploid × diploid; hexaploid × tetraploid. In each of these crosses some ovary development was observed within a few days after pollination, indicating that fertilization probably had occurred, but normal development was not maintained.

Seeds obtained from crosses between diploid × tetraploid were small and slightly shrivelled and failed to germinate. The hexaploid × tetraploid crosses resulted in a few normal appearing seeds which had a low germination. Seeds from other crosses germinated readily.

From the microscopic observations made on the sectioned ovaries of *A. strigosa* pollinated by *A. sativa* fertilization was obtained in 28.5 per cent of the pollinations. The hybrid embryo appeared normal at the early stage (2 days after pollination) and continued to develop quite normally from 4 to 8 days after pollination. Abnormalities in endosperm development were apparent at the 2-day stage and became progressively worse at the later stages. At 16 days after pollination, degeneration of both embryo and endosperm was almost complete. Although embryo culture was not attempted, it may be possible to grow excised embryos in nutrient media.

Fertility of Hybrids

The hybrids fell into one of two self-fertility groups. Hybrids between species having the same chromosome number were highly self fertile. Hybrids between species having different chromosome numbers were either highly or completely self sterile. Under field conditions hybrids of the latter group set seed in about 1 per cent of the florets. When bagged or grown in the greenhouse no seed was obtained.

Observations on the meiotic divisions of pollen mother cells revealed abnormalities in development among all highly sterile hybrids. The resulting quartets varied considerably in size, shape, and number of micronuclei. In spite of these abnormalities some of the pollen grains continued to develop to maturity. The proportion of apparently normal pollen at anthesis depended upon the direction of cross and upon the species involved. The proportion of apparently normal pollen was lower among hybrids between tetraploid and hexaploid species than between tetraploid and diploid species.

Sterility in the hybrid plant may also result from abnormalities in the formation of the female gametes. Backcrossing with pollen from either of the parental species was used to determine whether the female gametes of the hybrid were viable. Because the chromosome constitution of the parental male gametes differs from those of the hybrid female, incompatibility may be expected. Therefore, if viable seed is obtained, the female gamete is viable. If on the other hand no viable seed is formed, it may be due to an inviable female gamete or to incompatibility. Although seed set was generally low, backcrossed seed was obtained from hybrids of tetraploid \times diploid and tetraploid \times hexaploid species crosses only if the hybrid was used as the female parent.

Artificial Polyploids

Although successful doubling was obtained by several of the methods used, the vial method described by Bell (1) and Derman (5) was found to be most effective. A small vial of 0.05 per cent aqueous colchicine solution was inverted over the tillers of young plants decapitated at the 3- to 4-leaf stage. This method was used almost exclusively in treating the species hybrids to induce chromosome doubling. The following artificially induced polyploids were obtained: Autotetraploids of *A. strigosa* and *A. brevis* ($2n = 28$); Amphiploids *A. abyssinica* \times *A. sativa* ($2n = 70$); *A. abyssinica* \times *A. sterilis* ($2n = 70$); *A. abyssinica* \times *A. strigosa* ($2n = 42$). The seed characteristics of two of the amphiploids and their parents are shown in Figures 1 and 2. The amphiploid and normal tillers of the treated plants were morphologically similar in appearance. There was, however, an increase in size of pollen grains and stomata, an increase in the proportion of apparently normal pollen and subsequently in the self-fertility of amphiploid over the hybrid. The seed set per spikelet of the A_1 generation of the amphiploids ranged from 0 to 60 per cent of that of the parental species. These results differ from those reported by Lesik (10) whose amphiploids of the *A. sativa* \times *A. abyssinica* combination were up to 100 per cent fertile.

The production of seed varied considerably among the progenies of the amphiploids. The space planted progenies were grown under field conditions at Ottawa during the summer of 1954. The amphiploids tillered well and produced an abundance of spikelets but generally produced very little seed, due chiefly to a high degree of sterility. A rough estimate of the fertility of the amphiploids may be obtained by comparing their yields to those of the parental species. The average yield per plant among the parental species checks were: *A. strigosa*—14 grams; *A. abyssinica*—13 grams; and *A. sativa*—17 grams. A yield frequency distribution of A_2



FIGURE 1. Seed of parents and amphiploid of *A. abyssinica* \times *A. strigosa*. A: *A. abyssinica* CD 4550; B: Amphiploid of (CD 4550 \times CD 3820) ($2n = 42$); C: *A. strigosa* CD 3820.



FIGURE 2. Seed of parents and amphiploid of *A. abyssinica* \times *A. sativa*. A: *A. abyssinica* CD 4551; B: Amphiploid (CD 4551 \times Ajax) ($2n = 70$); C: *A. sativa* var. Ajax.

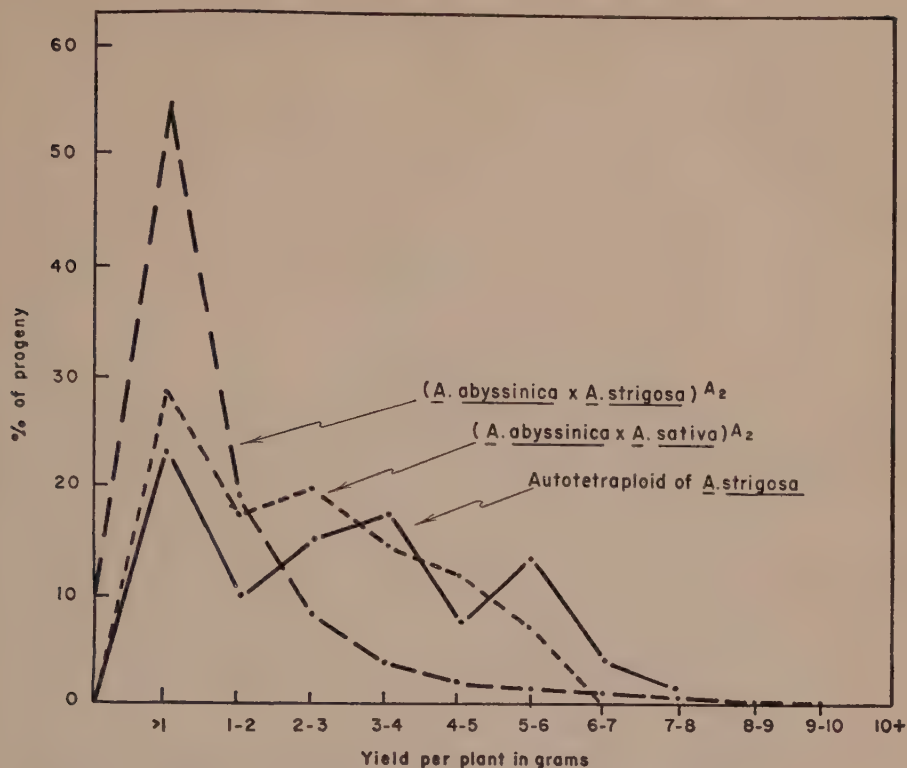


FIGURE 3. Frequency distribution of yields in grams per plant of A₂ progenies of three artificial polyploids.

progenies of 3 artificial polyploids is illustrated in Figure 3. The number of plants grown in each group was as follows: (*A. abyssinica* × *A. strigosa*) A₂—273; (*A. abyssinica* × *A. sativa*) A₂—65; autotetraploid of *A. strigosa*—49. Many of the amphiploids produced either no seed or less than 1 gram of seed while some showed moderate fertility but in no case did they approach that of the parental species. A higher proportion of the progenies of the amphiploid *A. abyssinica* × *A. strigosa* were in the low range of seed production (yielding less than 1 gram of seed) than progenies of the other two groups.

The compatibility of the autotetraploid (4x) of *A. strigosa** with species of the diploid, tetraploid, and hexaploid groups is illustrated in Figure 4. Triploid hybrids between the autotetraploid of *A. strigosa* and diploid species were easily obtained; they could be backcrossed to the diploid species and were partially self fertile. The low seed set obtained by backcrossing the autotetraploid (4x) with tetraploid species may be attributed to the difference in flowering-time between the two forms and to the poor pollen production of *A. abyssinica*. The hybrids could be backcrossed to either parent and were slightly self fertile. No difficulty was encountered in crossing the autotetraploid (4x) with the hexaploid species. Backcrossed seed was obtained using the hexaploid species as the male parent. The hybrids were completely self-sterile. Two hybrids seed were

*The autotetraploid of *A. strigosa* (4x) used was obtained from I. Nishiyama, Kyoto University, Japan.

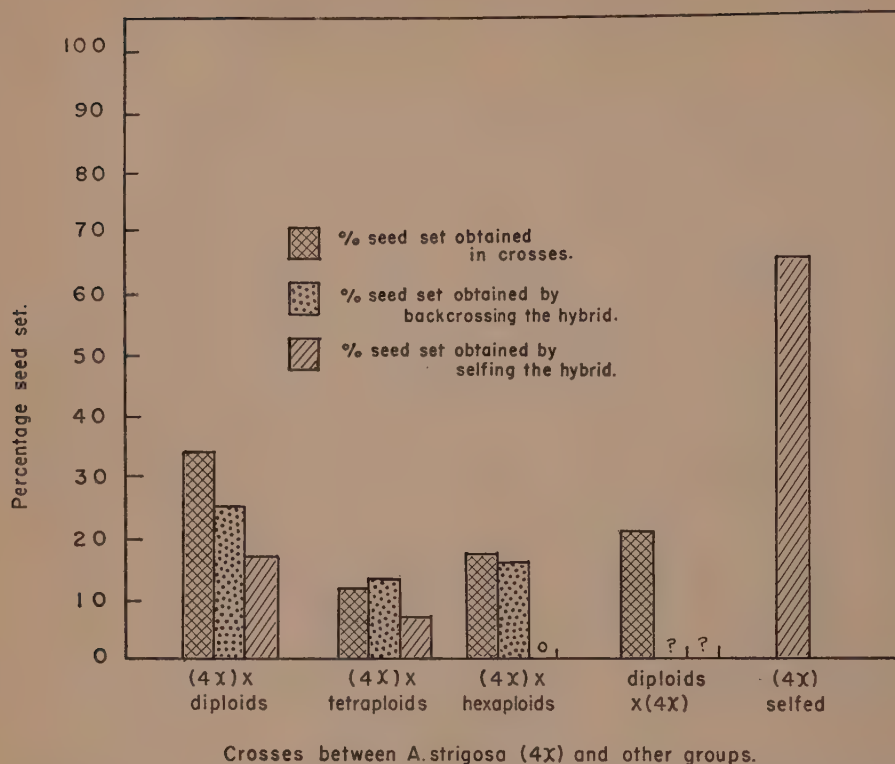


FIGURE 4. Cross compatibility relationships between the autotetraploid of *A. strigosa* (4x) and diploid, tetraploid and hexaploid species.

obtained by crossing the diploid female by the autotetraploid male (4x) of *A. strigosa*. The seeds were grown under greenhouse conditions which were not conducive to good seed set and no seed was formed. Backcrosses were not attempted. Under field conditions approximately 65 per cent of the florets of the autotetraploid (4x) set seed upon selfing. The seed of all crosses involving the autotetraploid of *A. strigosa* (4x) as the female parent germinated readily and produced vigorous hybrids.

In an attempt to transfer genes from the lower chromosome species to common oats, crosses were made between the autotetraploid of *A. strigosa* and *A. sativa*, and this hybrid backcrossed to *A. sativa* male. This may provide a means of transferring genes of the species *A. strigosa* to *A. sativa*. Among the factors which influence the success of such a project are: The amount of pairing between the chromosomes of the two species; the possibilities of obtaining individuals having a pair of chromosomes of *A. strigosa* added to the unaltered complement of *A. sativa* or substituting a pair of *A. sativa* chromosomes by a pair from *A. strigosa* as suggested by O'Mara (15); and the stability, vigour and fertility of the individuals obtained.

Another alternative was to cross the amphiploid of *A. abyssinica* × *A. strigosa* (synthetic hexaploid) to *A. sativa*, then to backcross the hybrid to *A. sativa*. Several such crosses were attempted in the greenhouse at



FIGURE 5. Seed of parents and hybrid of synthetic hexaploid (68-1-5) \times *A. sativa*. A: 68-1-5 (CD 4550 \times CD 3820); B: Selfed seed from F₁ hybrid (68-1-5 \times Victory); C: *A. sativa* var. Victory.

Ames, Iowa, during the winter of 1953-54. Five hybrid plants were obtained from 7 seeds sown in the field at Ottawa in the spring of 1954. The plants were vigorous, with coarse short straw and spikelets slightly larger than those of the *A. sativa* parent. Only one selfed seed, and no backcrossed seed, was obtained from these plants. The seed germinated but failed to reach maturity under greenhouse conditions during the winter of 1954-55. The seed characteristics of the parents and selfed seed of the hybrid are shown in Figure 5. Further crosses were attempted between the synthetic hexaploids and *A. sativa* and 31 hybrid seeds obtained. Backcrossing to the *A. sativa* parent will be attempted on these hybrids during the 1955 season.

As the amphiploid of *A. abyssinica* \times *A. strigosa* (synthetic hexaploid) is sufficiently compatible with *A. sativa* to produce a vigorous hybrid and an occasional selfed seed, there is hope that gene transfer from the lower chromosome species to *A. sativa* may be successful either by this method or through hybridization to the autotetraploid of *A. strigosa*.

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ALFALFA POLLINATION BY HONEY BEES ON THE REGINA PLAINS OF SASKATCHEWAN¹

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ABSTRACT

One, three and five colonies of honey bees per acre were distributed on duplicate 4-acre plots of Grimm alfalfa in the Regina Plains area of Saskatchewan in 1951, 1952, and 1953. Duplicate plots, isolated from honey bee colonies, were used as checks. Because of excessive competition from other flowering plants, chiefly wild mustard, not over 11 per cent of the field force of honey bees foraged the alfalfa even in early August after the competing bloom was appreciably reduced. Nevertheless, increased numbers of colonies per acre increased tripping. The degree of cross-pollination effected by honey bees was indicated by ratios for cross-, open- (honey bee), and self-pollination, respectively, of 2:1.4:1 for numbers of pods; 3:2:1 for seeds per pod; and 6:3:1 for seeds per floret. Yields of alfalfa seed obtained from the control and the 1-, 3-, and 5-colony-per-acre plots were 35, 45, 72, and 117 lb., respectively. The 5-colony-per-acre plots produced significantly more seed than the 1-colony-per-acre and control plots. These investigations showed that the bees will trip and cross-pollinate alfalfa under the climatic conditions of the area.

INTRODUCTION

Honey bees have been considered of negligible value for pollinating alfalfa in Western Canada (2, 3, 4, 6). They are important pollinators of alfalfa, however, in the southwestern United States (1, 5, 7, 8). Where honey bees have been used successfully, the need for large numbers of colonies has been emphasized. Because the use of heavy concentrations of honey bees has not been attempted in Canada, the effect of different numbers of colonies per acre on alfalfa seed yields was investigated in 1951, 1952, and 1953 in the Regina Plains area of Saskatchewan. This area, where nearly all of the land is used for cereal crops, was chosen because it was thought that native pollinators would not be a complicating factor and that competitive bloom could be eliminated readily.

EXPERIMENTAL PROCEDURE

Eight 4-acre plots, approximately 3 miles apart, were seeded to Grimm alfalfa in rows 36 inches apart in 1950. Plots A, F, G, and H (Figure 1) developed uneven stands and were reseeded in 1951.

Competitive bloom in the plots, chiefly wild mustard, was eliminated by spring cultivation, application of a selective herbicide, and hoeing as necessary.

Injurious insects, chiefly grasshoppers and lygus bugs, were controlled, except in 1953, when some damage by lygus bugs was evident.

¹ Joint contribution from the Divisions of Apiculture and Forage Crops, Experimental Farms Service, and the Entomology Division (No. 3358), Science Service, Ottawa, Canada.

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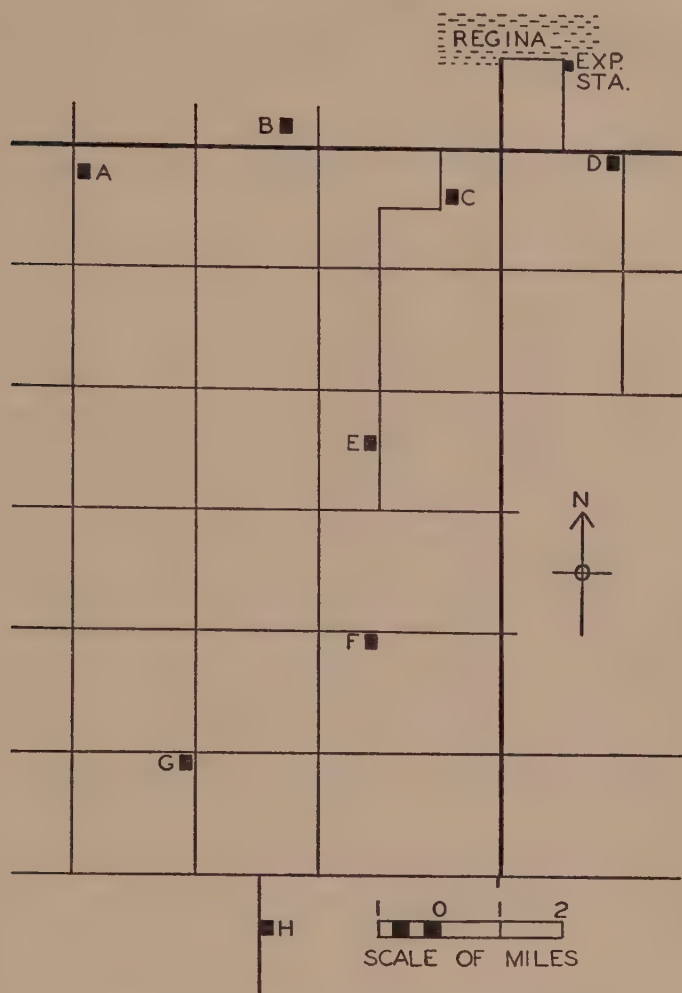


FIGURE 1. Location of alfalfa plots, Regina, Sask.

To reduce the almost negligible factor of native pollinators, *Bombus* spp. queens were collected in the spring from caragana hedges near the experimental plots.

Colonies of honey bees were distributed throughout the individual plots in the numbers indicated in Table 1, when the alfalfa was 5 to 10 per cent in bloom. The colonies were weighed immediately before transfer to the plots and prior to removal from the plot areas.

In addition to the plots with no bees, a further check on the effect of honey bees was made by placing three wire screen cages, $4 \times 4 \times 4$ feet, in each plot at the commencement of bloom to exclude all pollinators.

In order to determine the number of honey bees actually foraging the alfalfa, one-minute counts were made in 20 one-square-yard areas of each plot during the afternoons of July 22 and 29, and August 14, 1953.

TABLE 1.—ASSIGNMENT OF DIFFERENT CONCENTRATIONS OF HONEY BEES TO THE VARIOUS PLOTS, 1951-1953

Year	5 colonies per acre	3 colonies per acre	1 colony per acre	No honey bees
1951 ¹	D, E	C, H	B, G	A
1952 ¹	A, E	D, G	B, F	C, H
1953 ²	G, H	E, F	A, B	C, D

¹ Treatments assigned at random.² Treatments assigned to prevent bee visitations from plots containing 5 and 3 colonies per acre.

Counts of freshly tripped florets per 100 racemes were made at random on four dates in 1952, to determine the relative amounts of tripping in different plots. Counts were made between 1.30 p.m. and 4.30 p.m. each day.

In order to test the effectiveness of honey bees in cross-pollinating alfalfa, comparative data on number of pods formed, number of seeds per pod, and number of seeds per tripped flower were recorded from open- (honey bee), cross-, and self-pollinated flowers in 1952 and 1953. In 1952, 37 florets observed to be tripped by honey bees were tagged; on the same plants 91 florets were cross-pollinated and 82 florets were self-pollinated. In 1953 comparative data were recorded from 10 open-pollinated, 10 cross-pollinated, and 10 self-pollinated florets in each of 40 plants in plot G, where the absence of native pollinators ensured that tripping could be attributed to honey bees.

Twenty plots, each 8 × 3 feet in size, were harvested at random from each area to provide an estimate of seed yield. The caged alfalfa areas were harvested and yields determined.

RESULTS AND DISCUSSION

Precipitation data for the pertinent months of each year of the investigation are compared with the long-term averages in Table 2.

TABLE 2.—PRECIPITATION IN INCHES DURING MAY, JUNE, JULY AND AUGUST, 1951-1953, AND 64-YEAR MEAN¹

Year	May	June	July	August
1951	0.26	4.79	1.27	3.59
1952	0.50	3.91	2.41	2.72
1953	2.68	4.05	3.48	0.92
64-year mean	1.71	3.20	2.30	1.77

¹ Annual Meteorological Summary for Regina, Sask., 1953. Meteorological Division, Canada Department of Transport.

TABLE 3.—AVERAGE NUMBERS AND PERCENTAGES OF FIELD FORCE OF HONEY BEES FORAGING THE ALFALFA ON JULY 22 AND 29 AND AUGUST 14, 1953, IN PLOTS WITH DIFFERENT NUMBERS OF COLONIES PER ACRE

Colonies per acre	Number of honey bees per square yard	Estimated percentage of field force ¹
1	0.24	11.6
3	0.34	5.5
5	0.90	8.7

¹ Based on a normal field force of 20,000 bees per colony, of which one-half would be foraging and the other half in flight to or from the hive.

TABLE 4.—NUMBERS OF FRESHLY TRIPPED FLOWERS PER 100 RACEMES ON FOUR DATES IN 1952

Date	Temperature, °F.	Wind velocity, miles per hr.	Colonies per acre				Average
			0	1	3	5	
July 30	87	3	31	59	70	91	63
August 6	78	17	19	36	50	71	44
August 12	85	5	85	80	84	145	98
August 18	79	7	93	121	121	155	122
Average.....			57	74	81	116	

Data on numbers of bees foraging the alfalfa, amount of tripping, amount of cross-pollination, alfalfa seed yields, and honey production are presented in Tables 3 to 7.

Excessive rainfall in the Regina area during June, 1951 and 1952, and May and June, 1953, greatly stimulated the growth of wild mustard and interfered with its control. The abundant competitive bloom throughout the area attracted so many honey bees that only a small portion of the field force foraged the alfalfa (Table 3).

The effect of competitive bloom was evident in the honey yields (Table 7). With the exception of 1952, colonies in the 5-colony-per-acre plots did not yield significantly less than those in the 1- or 3-colony-per-acre plots.

Counts of tripped florets (Table 4) revealed increased numbers in the plots with more colonies of bees though the increases were not proportional. These data also showed an increase in tripping as the season advanced and competitive bloom decreased. The reduced amount of tripping recorded on August 6 was probably due to the comparatively high wind velocity which retarded honey bee flight on that date.

TABLE 5.—PERCENTAGE OF PODS FORMED, NUMBERS OF SEEDS PER POD, AND SEEDS PER FLORET TRIPPED, FROM FLOWERS POLLINATED IN VARIOUS WAYS

Year	Type of pollination	No. florets tripped	Percentage pods formed	No. seeds per pod	No. seeds per floret tripped
1952	Cross	91	74	5.1	3.8
1952	Open (honey bee)	37	78	4.8	3.7
1952	Self	82	24	3.4	0.8
1953	Cross	400	80	5.2	4.0
1952	Open (honey bee)	400	57	3.5	2.0
1953	Self	400	39	1.7	0.7
L.S.D. (1953 data only) at 1% level.....				0.9	0.9

TABLE 6.—ALFALFA SEED YIELDS IN POUNDS PER ACRE

Colonies per acre	1951	1952	1953	Average
Caged areas (all plots)	17	10	12	13
0	37 ¹	56 ²	13	35
1	44	65	26	45
3	49	114	54	72
5	52	151	147	117
L.S.D. 5% level.....				52

¹ Some *Bombus* spp. and *Megachile* spp. present.² Honey bee numbers present approached those in plots with 1 colony per acre, due to proximity of a plot containing 5 colonies per acre.

TABLE 7.—HONEY YIELDS IN POUNDS PER COLONY

Colonies per acre	1951	1952	1953	Average
1	173	39	96	103
3	120	45	110	92
5	128	— 4 ¹	96	73
Average	140	27	101	
L.S.D. at 5% level.....				49 lb.

¹ Significantly different from the 3- and 1-colony-per-acre yields in 1952 at 5% level.

The data on the effectiveness of different types of pollination gathered in 1952 prompted a more detailed examination in 1953 (Table 5). Statistical analysis of the 1953 data showed that open- (honey bee) pollinated flowers formed more pods and set more seeds per pod and per tripped floret than self-pollinated flowers, but less than artificially cross-pollinated flowers.

Analysis of the yield data presented in Table 6 showed that a significant increase occurred only between plots with 5 colonies and 1 colony per acre and the check plots. In the 3-colony-per-acre plots the yields were similar to those of the 1-colony-per-acre and check plots, except during 1952, when they were nearly double.

The increased tripping that occurred after August 6 (see Table 4) resulted in a high percentage of immature seed due to early frosts which are normal for this area.

CONCLUSIONS

These investigations show that honey bees will trip and cross-pollinate alfalfa under the climatic conditions of the Regina Plains area of southern Saskatchewan. It was also shown that a higher seed yield was obtained when a greater number of colonies per acre were used. The results of this study suggest that the increase in alfalfa seed yield due to honey bee pollination would appear to be insufficient to cover the cost involved.

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THE X-RAY SENSITIVITY OF CUTTINGS OF CREEPING-ROOTED ALFALFA¹

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ABSTRACT

A method for the determination of radiosensitivity of alfalfa is presented. Alfalfa stem cuttings were exposed to 9 dosage levels of X-radiation, namely, control (no radiation), 500r, 1000r, 2000r, etc., to 7000r, subsequent to the initiation of root primordia at the base of each cutting.

A reduction in the lengths and the number of roots per cutting was the initial manifestation of sensitivity to X-radiation. Complementary information was contributed by axillary bud growth and leaf anomalies. No irradiation effects on root or axillary bud growth were visible in cuttings exposed to the 1000r level or less. Growth of both roots and axillary buds was inhibited at dosage levels of 5000r or more. Adventitious stems developed on the roots at the base of cuttings if the rooting portion were shielded from X-radiation.

INTRODUCTION

Alfalfa is a crop of considerable economic importance in which agronomic improvements still are to be made (1, 4). Irradiation studies were initiated in creeping-rooted alfalfa on the assumption that desirable mutations might be induced in this material as has been the case in other crops and varieties in which ionizing radiations were used as a means of inducing changes of agronomic value (3, 5, 6, 8).

Investigations conducted on creeping-rooted alfalfa, employing ionizing radiations as a means of inducing heritable changes, involve, initially, a determination of the type of plant material most suitable for transmitting changes to succeeding generations and dosage levels of radiation required to induce mutants for selection purposes.

Alfalfa presents a number of problems in so far as irradiation studies are concerned. It is a cross-pollinated, polyploid crop with an inherently variable seed population, thus making it difficult to determine the degree of natural and induced variability. This difficulty might be circumvented somewhat, because alfalfa is a perennial crop and readily propagated vegetatively, by exposing stem cuttings not only to different levels of radiation but also to various radiation treatments, thus permitting a comparison of the progeny from irradiated and unirradiated cuttings of each individual clone.

The axillary buds on each cutting provide potential regions for induced variability in the plant material. Numerous adventitious stems develop on the roots of creeping-rooted alfalfa (4, 7). Thus, additional sources of potential variability accrue when adventitious stems are initiated on roots which have been exposed to radiation treatments.

Information is presented on the sensitivity of a creeping-rooted alfalfa clonal line to different levels of X-radiation and the techniques used in making these determinations.

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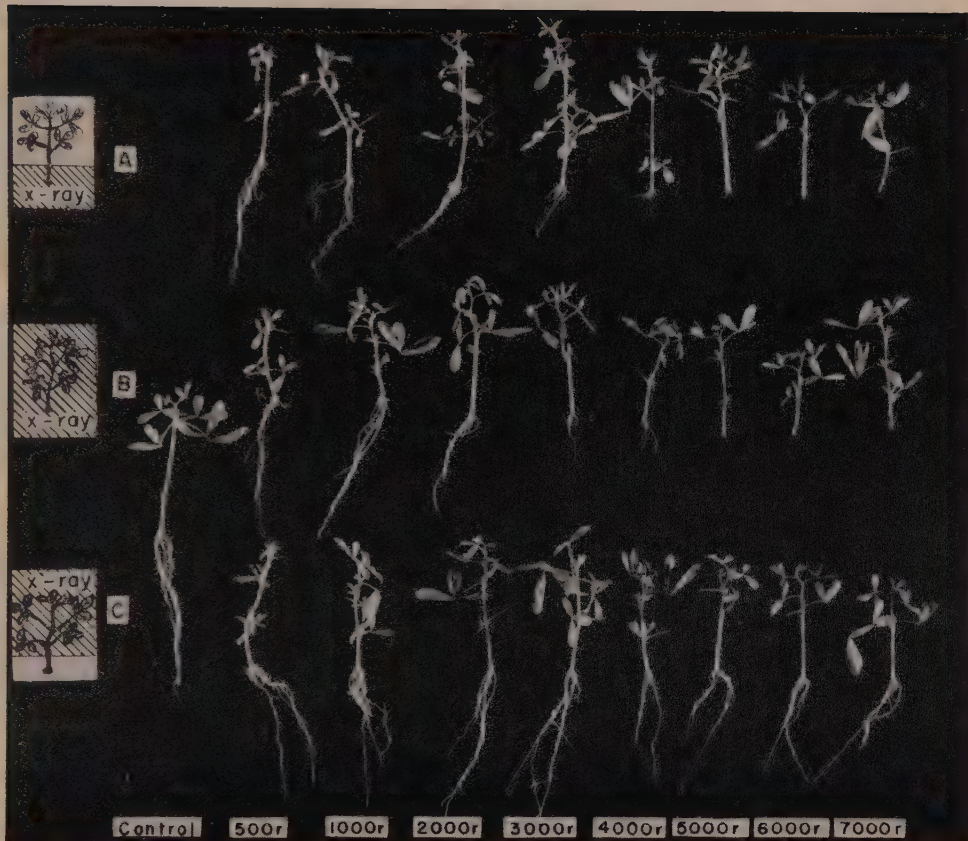


FIGURE 1. An illustration of root length on cuttings 11 days after exposure to different treatment levels of X-radiation (A—rooting portion exposed; B—entire cutting exposed; C—rooting portion shielded).

MATERIAL AND METHODS

Stem cuttings from one creeping-rooted clone, selection 25029, were used as the source of material for the experiment. This clone is a selection from the creeping-rooted progenies of an interspecific cross involving Ladak (*Medicago sativa* L., sometimes referred to as *M. media* Pers.) and Siberian (*M. falcata* L.). The clone is a profuse creeping-rooted plant, i.e., numerous stems develop on the roots. The stems thus produced are referred to as "adventitious".

The cuttings ranged from 6 to 8 centimetres in length and involved from one to three nodes of a stem. The stem cuttings were placed in moist vermiculite in a humid greenhouse for 4 days, until root primordia were initiated as evidenced by a slight swelling at the base of the cuttings, at which time they were exposed to the following treatments:

- A. Rooting portion of cutting exposed to X-rays and stem portion shielded by a half-inch of lead.
- B. Entire cutting exposed to X-radiation.
- C. Stem portion exposed to X-radiation and rooting portion shielded.

Within each treatment 9 dose levels were used, namely, control (no radiation), 500r, 1000r, etc., to 7000r. Each exposure treatment within a level contained 26 cuttings, giving a total of 624 cuttings exposed to X-radiation.

After exposure to X-rays, the cuttings were returned to the vermiculite. Data on the number and total length of growth of roots per cutting were taken at 2-day intervals until definite irradiation effects were noted. Eleven days after exposure these effects were sufficiently pronounced and the final measurements on root performance were recorded.

The cuttings with roots were planted in soil. Observations were made on the rate of growth of axillary buds and the various leaf forms or anomalies which were visible as axillary buds gave rise to the new growth or stems of the cuttings. Data on the height of this new growth were recorded 54 days after exposure of the cuttings to X-radiation.

The data on root performance are presented as the average of the length of root growth and the number of roots of the 26 cuttings in each treatment level. The data on the axillary bud growth are presented as the average height of the new growth of cuttings in each treatment level.

The X-rays used in this study were generated with a G.E. Maxitron facility operated at 250 KVP and 30 ma. with 1 mm. aluminum filtration. The plant material received about 580r per minute at its surface as measured by a Victoreen Integron.

EXPERIMENTAL RESULTS

Root Length

A somewhat consistent reduction in the average length of root growth occurred when the dose level of X-radiation was increased if the rooting portion of cuttings were exposed to X-rays (Figures 1 and 2). The average root length per cutting in each treatment level, 11 days after exposure, decreased from 6.6 cm. for the unirradiated control to 0.3 cm. and 0.5 cm. at 7000r for the cuttings in treatments A and B respectively. The rate of root growth was reduced markedly at dose levels of 5000r or more. If the

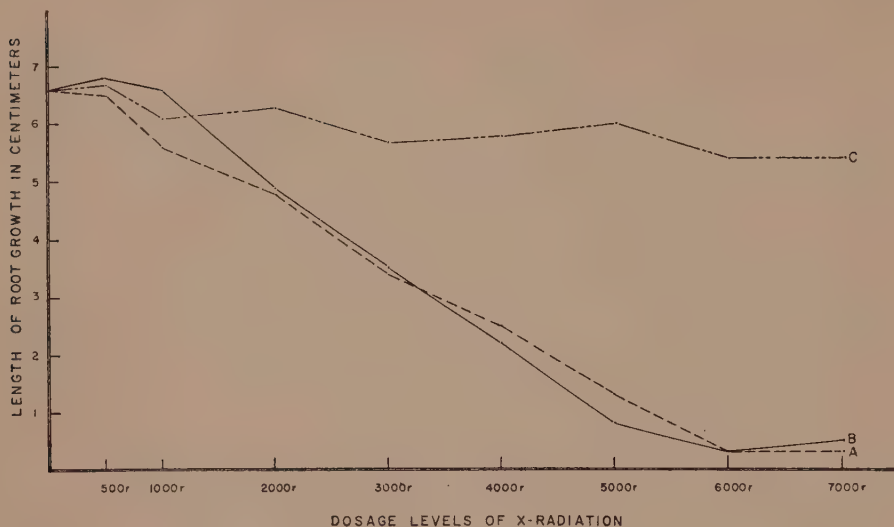


FIGURE 2. The average length of root growth per treatment level 11 days after exposing the cuttings to X-radiation (A—rooting portion exposed; B—entire cutting exposed; C—rooting portion shielded).

rooting portions of the cuttings were shielded (treatment C), root growth did not appear to be affected by exposing the upper portion of the cuttings to the different levels of X-radiation (Figures 1 and 2).

Number of Roots

There was a progressive reduction in the average number of roots per cutting in treatments A and B when the rooting portion was exposed to the dosage levels from 3000r to 7000r (Figure 3). The number of roots ranged from an average of 7.0 per cutting for the control to an average of 1.3 and 1.1 at 7000r for treatments A and B respectively. On the other hand, when the rooting portion was shielded from X-radiation (treatment C), the average number of roots per cutting was comparable with that of the unirradiated control (Figure 3).

Axillary Bud Growth

Growth of axillary buds was evident by 21 days. By 54 days subsequent to X-radiation, the height of this new growth was considerably less for all treatments at the 4000r level than at lower levels of X-radiation (Figure 4). No axillary buds developed if they were exposed to the 6000r and higher levels of X-radiation. At the 5000r level, however, eight cuttings in treatment A (stem portion shielded) had sufficient root development to enable the axillary buds to grow; no cuttings survived in treatment B; and two cuttings in treatment C showed very limited axillary bud growth.

Leaf Anomalies

No visible effects on leaflet development were noticed at the 500r and 1000r level of X-radiation. At 2000r, however, the edge of the leaflets was slightly lobed (Figure 5a). As the level of X-radiation was increased, effects were more pronounced (Figure 5b). There was an over-all diminu-

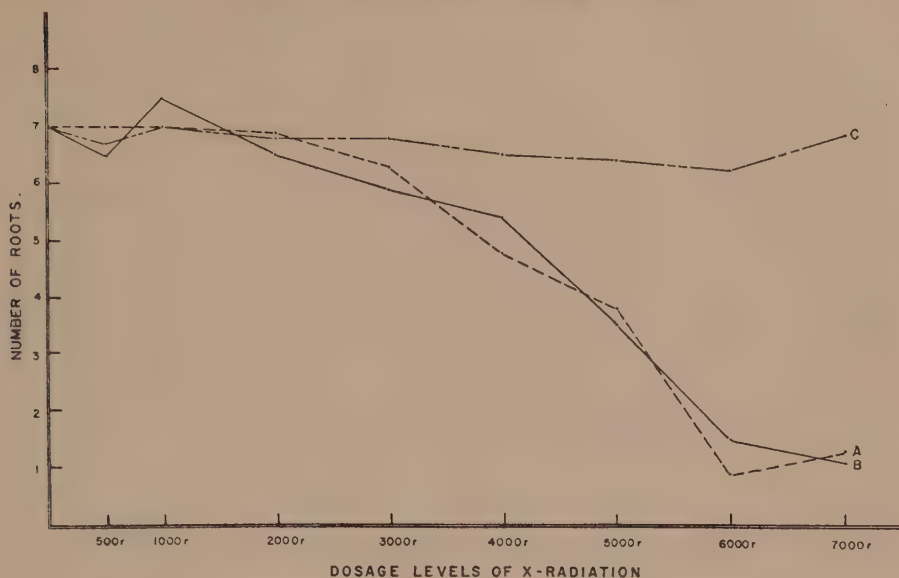


FIGURE 3. The average number of roots per treatment level 11 days after exposing the cuttings to X-radiation (A—rooting portion exposed; B—entire cutting exposed; C—rooting portion shielded).

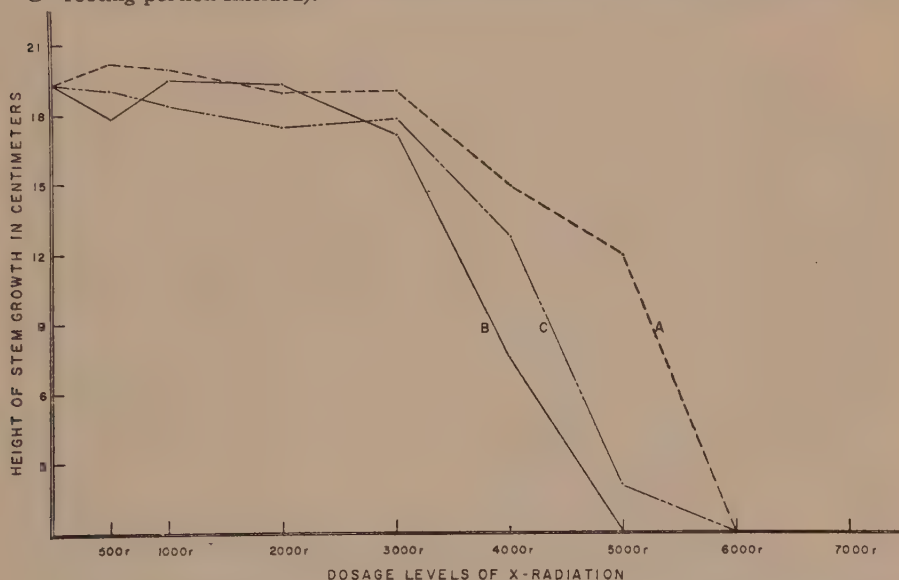


FIGURE 4. The average height of new growth per treatment level 54 days after exposing the cuttings to X-radiation. (A—rooting portion exposed; B—entire cutting exposed; C—rooting portion shielded).

tion in size of all leaves. In some there was evidence of inhibited development of one or two leaflet primordia. Others showed a creped appearance and frequently exhibited chlorotic streaking. However, as the axillary buds continued to grow, the leaves formed at a later stage were normal in appearance.

Adventitious Growth

No adventitious stems were observed on the roots of cuttings exposed to X-radiation in treatments A and B or in the control cuttings. Numerous adventitious stems formed on the roots produced by the cuttings in treatment C which received a dosage level of 2000r or more (Figure 6). Adventitious stems were produced in a shorter period of time following exposure to the 6000r and 7000r dosage levels than to the 2000r to 5000r levels. Adventitious stems had grown to a height of three or four centimetres by 54 days following exposure to the 6000r and 7000r levels; but 84 days had passed before similar adventitious growth was visible in the cuttings of treatment C exposed to the 2000r to 5000r levels of X-radiation. There was no axillary bud growth at the higher levels of exposure, but the cuttings retained their deep green colour during the entire period.

It was of interest to note that roots developed at the base of the shielded section of stem cuttings in treatment A in which the basal portion was subjected to lethal levels of X-radiation (Figure 7). The shift in region of rooting occurred when the cuttings were retained under conditions favourable for rooting. This rooting response was similar to that reported by Christensen (2) following localized irradiation of a small length of stem of intact seedlings from a number of species.

DISCUSSION AND CONCLUSIONS

The retarding effect of X-radiation on root growth of rooting alfalfa stem cuttings was noted to be a method for determining radiosensitivity of both root and stem primordia. Complementary information on radiosensitivity was contributed by the number of roots, the rate of growth of axillary buds, and by various leaf anomalies.

The 4000r level of X-radiation was the highest level at which there was growth of alfalfa cuttings under the conditions which prevailed during the investigation. This was apparent as no cuttings in treatment B (entire exposure) survived at 5000r. Shielding the rooting portion (treatment C) at this level of X-radiation did not appear to change the level of radiosensitivity of the axillary buds as very limited new growth was evident in only two cuttings. Conversely, shielding the stem portion (treatment A) did not affect the level of radiosensitivity of the roots. The axillary bud growth of cuttings subjected to 5000r in treatment A could be attributed, probably, to the ability of alfalfa cuttings to persist even on a very limited root system rather than to an increase in radioresistance of the roots.

Physiological effects of radiation were manifest at the 2000r level of X-radiation. Leaf anomalies were visible at this level and were not detected at lower doses. Furthermore, root growth was retarded in comparison with that of either unirradiated root primordia or of those which received less than 2000r.

In this experiment 2000r was the lowest level at which physiological effects of radiation were visible, and 4000r was the highest level of radioresistance of the alfalfa cuttings. The range within which mutations could be induced in irradiated alfalfa cuttings appears to lie between 1000r and 3000r. This range is based on root length and number, leaf



FIGURE 5a Leaf anomalies from cuttings of a clone 40 days after exposing the cuttings to the 2000r level of X-radiation.



FIGURE 5b Leaf anomalies selected from cuttings of a clone 40 days after exposing the cuttings to the 3000r and 4000r levels of X-radiation.



FIGURE 6 Cuttings from treatment C showing the regions of initiation (arrow) of adventitious stems (S) on roots (R). (54 days after exposure to X-radiation).



FIGURE 7 An illustration of root development on cuttings exposed to 7000r in treatment A.

anomalies and height of axillary bud growth of cuttings exposed under the conditions of growth similar to those which prevailed in this experiment. It is possible that radiation dosages other than those established by this study might be useful if there is a change in certain factors, whether these be age of cutting, degree of wilting, or rate of growth.

Cuttings survived dosage levels of 6000r and 7000r if adventitious growth developed from the unexposed regions. The centre of growth shifted to the region proximal to the X-rayed portion of cuttings in both treatment A (stem shielded) and treatment C (root shielded). The shift in region of growth was most striking in the cuttings of treatment C which received high doses of X-radiation. Even in preliminary experiments the same shift in growth region was noted when the stem portion was subjected to 18,000r. If there is a substance in the stem portion responsible for rooting, it apparently was not destroyed by the dosage levels used in this experiment. Although the exposed stem portion (rooting portion shielded) showed no visible signs of growth, the cuttings carried on the processes necessary for their roots to continue to grow and to develop adventitious stems.

It appears that X-irradiation has the effect of indirectly stimulating adventitious stem initiation on roots if the stem portion of cuttings is exposed to dosage levels of 2000r or more. Adventitious stem development on roots of creeping-rooted alfalfa is a natural phenomenon, but the interval of time in which profuse adventitious stem growth occurred following irradiation is very much less than the period required under natural conditions of growth (4, 7). Thus, in plants such as creeping-rooted alfalfa which possess the inherent characteristics of initiating adventitious growth, entire cuttings should be exposed to ionizing radiations if the seed from the resultant plants is to be used for a study of mutations. Whether or not alfalfa plants which normally do not develop adventitious stems on their roots will be stimulated to do so following localized irradiation, remains to be determined.

The rate of root and axillary bud growth of cuttings within each treatment level was somewhat variable. Part of this variation might be attributed to the effects of X-irradiation and part is a characteristic phenomenon of rooting alfalfa cuttings.

Further investigations should give some indications of the type of mutations to expect, the dosage levels and number of radiation treatments for inducing agronomically valuable mutations, and the factors which could influence the radiosensitivity of cuttings of creeping-rooted alfalfa. Although from 1000r to around 3000r were the dosage levels of X-radiation considered optimum for cuttings under the growth conditions used in this experiment, other factors such as type of plant material under investigation, growth rate, degree of wilting or dryness, age, dormancy, etc., could necessitate a marked shift in dosage levels.

ACKNOWLEDGEMENTS

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GREENHOUSE AND FIELDPLOT STUDIES ON VARIETAL REACTIONS TO BARLEY LEAF RUST¹

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ABSTRACT

Dwarf leaf rust of barley caused by *Puccinia hordei* Otth. occurs frequently as rather severe local epidemics, and approximately every third year in epidemics covering large sections of the barley growing regions of North America, particularly east of the Rocky Mountains. It causes considerable damage to the crop by lowering the yield, grade, and malting quality. Of the 324 varieties and selections tested in the seedling stage, 99 were immune or at least moderately resistant to each of the rust races used. Another group of 122 were resistant to some races and susceptible to others. A total of 100 barleys were tested for adult plant reaction, in uniform rust observation nurseries in the United States, during a 20-year period. Six of these were consistently resistant to leaf rust. At Winnipeg, of 234 varieties and selections, subjected to artificially induced epidemics during four years, 36 never developed more than a trace of dwarf leaf rust. Varieties that were resistant in the seedling stage were also resistant in the adult plant stage, whereas some that were susceptible in the seedling stage became resistant in the adult plant stage.

INTRODUCTION

In their 1952 publication, the authors (4) presented a brief review of the history of barley leaf rust epidemics in the United States and Canada. Their principal concern, however, was with co-ordinating and consolidating the rust's physiologic races which had been variously designated in the published reports of other workers (1, 2, 3, 5, 8, 9, 10, 12), as well as in their own theretofore unpublished records (4). To achieve this objective it was necessary to test the reactions of seedlings of all readily available barley varieties, which at one time or another had been used as differential hosts, to as many isolates of the dwarf leaf rust as conditions and facilities permitted. As a result of these tests it became possible to select a workable set of critical differentials. These, in turn, facilitated the determination of the occurrence and distribution of the so-called consolidated, or integrated, barley leaf rust races in Canada and the United States.

Concurrent with and subsequent to these determinations, testing of many additional varieties and selections of barley was carried out in both the seedling and maturing stages, the former in the greenhouse and the latter in field-plots. Field tests in the United States continued from 1935 to 1954, inclusive, while in Canada tests were made during four years, 1944 to 1947. Greenhouse studies were of longer duration in Canada and of shorter duration in the United States. These tests constitute the subject of the present paper. It is hoped that the results obtained may prove of value to plant breeders and plant pathologists in their effort to control or reduce the damage caused by recurrent barley leaf rust epidemics before

¹ Co-operative investigations of Field Crops Research Branch, Agricultural Research Service, United States Department of Agriculture; Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota; and the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario. (Contribution No. 1482).

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their destructiveness becomes too great and too general. Severe leaf rust epidemics in the barley-growing regions east of the Rocky Mountains have, during the past two decades, occurred once every third year; very severe local epidemics have, on the average, occurred about every other year.

Newton, Peturson, and Meredith (7) have demonstrated that the damage from severe leaf rust infection is not inconsiderable, especially with regard to yield, grade, and malting quality of some of the 6 barley varieties involved in their study. They have summed up the highlights of their findings in the following words: "Leaf rust reduced the grade of O.A.C. 21 and Mensury by one commercial grade, and caused statistically significant reductions in the yield, bushel weight, and kernel weight of all the varieties tested except Mensury. It adversely affected the value of all the varieties for malting purposes by reducing the percentage of heavy-grade kernels. The nitrogen content and the wort nitrogen content were reduced by leaf rust, but the malt extract and diastatic powers were not greatly affected".

More than a quarter-century ago, Waterhouse (12) was first to report the results of testing barley seedlings for their leaf rust reaction. He found that 25 varieties and selections out of a total of 117 were extremely, and 10 moderately, resistant to the one Australian race involved in that experiment.

Three years later, Hey (2) published the results of his tests with 273 varieties, wherein six German races were involved. Only a single variety was uniformly highly resistant to all six races; one other variety was resistant to three of the races; two more were resistant to two races; and an additional nine varieties were resistant to one race.

The following year, Mains and Martini (6) reported on their field and greenhouse tests at Purdue University with some 660 varieties and selections of barley in 1924. Over one-third of these varieties they again tested in the greenhouse in 1925. More seedling tests were carried out with a smaller number of varieties during 1926-1929 and additional field tests were made during 1927-1929. Only a score or so of the varieties were consistently highly resistant under field and greenhouse conditions, in both the seedling and adult stages.

Further German studies were reported on briefly by Straib (11) in 1937. He had tested a total of 508 varieties and selections of barley to eight parasitic races of leaf rust. He found that some varieties, which had been susceptible as seedlings in the greenhouse, resisted infection in their adult plant stage when grown in the field.

The present writers observed similar maturative reactions to leaf rust among a number of barley varieties and selections, in both the greenhouse and the field. However, they have not so far encountered the reverse phenomenon, that is, varieties or selections highly resistant to certain physiologic races in the seedling stage becoming very susceptible, or even partially so, to the identical races in their maturing stage.

METHODS, MATERIALS, AND SCOPE OF INVESTIGATION

The studies on seedling reactions to individual physiologic races of *Puccinia hordei*, the leaf rust parasite, were made in the greenhouse at Winnipeg and St. Paul. From 15 to 30 seedlings of a desired variety or selection were grown in 4-inch earthenware pots. When the seedlings were

about a week old, and their primary leaves were well developed, they were inoculated with a specified rust race. The inoculations were made either by rubbing gently, though firmly, each leaf between two spore-covered fingers; by shaking heavily rusted plants over the seedlings; or by brushing severely infected plants several times over the seedlings. The inoculated seedlings, after having been incubated in moist chambers for 24 to 48 hours, were removed to compartments on the greenhouse benches and kept there at a temperature fluctuating between 18° and 20° C. The tests were often made in duplicate and, where the results were uncertain, the tests were repeated two or more times.

Altogether 324 varieties and selections of barley were tested in the seedling stage at the two rust research laboratories. There was some duplication in the barleys used at the two places. Similarly, some of the leaf rust races used at one place were also used at the other. A total of 218 varieties were tested at St. Paul, each with from 3 to 21 races, constituting 2132 separate tests. As many as 194 or 89.0 per cent of these barleys were tested with eight or more different races. At Winnipeg, a total of 156 barleys were tested, each with from 1 to 12 races, resulting in 708 separate tests. In this instance, 21 of the barleys, or 13.5 per cent, were tested with eight or more different races. Barring races 41 and 42, all the other leaf rust races thus far isolated from North American collections were included in the seedling tests. In addition, race 50, which originated as a mutant, was also used. Observations on seedling reaction were made 2-3 weeks after inoculation, depending on prevailing environmental conditions.

The reactions of adult plants were studied in two types of field tests. One of these consisted of a series of uniform rust observation nurseries maintained in different parts of the United States during the 20-year period, 1935-1954, inclusive. The barley varieties grown in these nurseries depended for leaf rust infection on naturally occurring epidemics. The uniform nurseries that were most severely affected by leaf rust were chosen as expressive of the varietal reactions during any specified year. In Canada, two or more varieties of barley have been grown in rust nurseries throughout the country since 1942, but this has been mainly for the purpose of determining the presence and the severity of leaf rust and other diseases in the country and also to obtain disease specimens from different parts of the country. The study on reactions of adult plants to leaf rust was confined to a nursery at Winnipeg, which was maintained during the 4-year period of 1944-1947. Here the epidemics were induced by artificial inoculations with uredial composites of as many different races as were available during a given season. Since reactions in the first 2-year period differed on some varieties from those during the last 2 years, biennial averages were considered for the purpose of comparison. Detailed notes were taken in both types of field tests at the optimum development of the leaf rust epidemics concerned.

EXPERIMENTAL RESULTS

Over 30 per cent of the varieties and selections of barley tested in the seedling stage at St. Paul, Minn., or at Winnipeg, Man., and in some cases

at both centres, were variously resistant to each of the leaf rust races used. However, among the 99 varieties so resistant, 9 had been tested with only 2 races; 1 with 3 races; 3 with 4 races; 20 with 5 races; 1 with 7 races; 4 with 8 races; 52 with 9 races; 1 with 11 races; 4 with 12 races; 3 with 14 races; and only 1 with as many as 19 races. Exactly two-thirds of these barleys were resistant to at least 7 of the consolidated races, besides one or more of the subordinate races used in a number of these tests. Only one-eleventh of the 99 varieties were resistant to 11 or more consolidated races and any of their subordinates. The dominant seedling reactions are recorded in Table 1 as either resistant or susceptible. The column headed "Resistant" lists those races which produced infection types 0, 1, or 2; the column captioned "Susceptible" includes the races which produced infection types X, 3, or 4. Varieties susceptible to all the races tested are listed in the text.

TABLE 1.—COMPARATIVE SEEDLING REACTION OF DIFFERENT VARIETIES AND SELECTIONS OF BARLEY TO VARIOUS CONSOLIDATED PHYSIOLOGIC RACES OF LEAF RUST*

Varieties concerned		Reaction to specified races	
Name	C.I. No.**	Resistant	Susceptible
Abacus	1088	2, 4, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	
Abyssinian	2192	1, 2, 4, 11, 37, 38, 40, 44, 45, 49, 51, 52.	39, 47; also L-21, a subordinate race of UN-44.
Aethiops	2208	1, 4, 5, 37.	
Afghan 2	6366	1, 3, 4, 5, 16, 38, 40, 43, 44, 45, 46, 47, 49, 50.	2, 11, 32, 39, 48.
Algerian	1179	4, 37.	1, 5.
Apsheron	5557	1, 4, 11, 44, 45.	
Arequipa	2329	1, 2, 4, 37, 39, 44, 45, 47, 49, 51, 52.	38, 40; also C-9, C-13, L-21, and L-23, subordinates of UN races 4, 2, 44, and 45, respectively.
Austral	6483	5, 37, 40, 46, 48; also B-4, C-9, C-16, L-77, all subordinates of UN-4; and C-13, a UN-2 subordinate.	1, 2, 3, 4, 11, 32, 34, 35, 36, 38, 39, 43, 44, 45, 47, 49, 50, 51, 52; also C-23, a subordinate of UN-37.
Bark	2793	4.	37; also C-9, a subordinate race of UN-4.
Barley 305	6015	2, 4, 38, 39, 40.	
Batna	3391	2, 4, 38, 39, 40.	
Bavaria	6395	1, 2, 4, 11, 16, 32, 35, 37, 38, 39, 43, 44, 46, 47, 48, 49.	3, 5, 34, 36, 40, 45, 50.
Beecher	6566	4.	37; also C-9, a subordinate of UN-4.
Beldi Giant	2777	4.	37.
Berg	6486	1, 2, 4, 11, 16, 34, 35, 37, 38, 40, 43, 44, 45, 48.	3, 5, 32, 36, 39, 46, 47, 50; also C-13, a UN-2 strain.
Blackhull	878	4.	1, 5, 37; also C-9, a subordinate of UN-4.
Black Russian	705	51.	2, 4, 37, 44, 45, 47, 49, 52.
Black Russian	2202	51.	1, 4, 11, 37, 44, 45, 47, 49, 52.
Blue Hulless	4848	4, 37.	C-9, a UN-4 subordinate.
Boehmes Beardless	2203	1, 4, 5, 37.	

* The Arabic numerals in the Resistant and/or Susceptible columns represent consolidated or "UN" races; those prefixed by a particular capital letter are subordinates or biotypes of the consolidated races. As explained elsewhere (4) the initials prefixed to the latter signify the respective authorities of the unconsolidated races in question: thus, B = Brown, C = Cherewick, L = Levine, and W = Waterhouse.

** C.I. stands for cereal introduction number, U.S.D.A.

TABLE 1.—COMPARATIVE SEEDLING REACTION OF DIFFERENT VARIETIES AND SELECTIONS OF BARLEY TO VARIOUS CONSOLIDATED PHYSIOLOGIC RACES OF LEAF RUST—*Continued*

Varieties concerned		Reaction to specified races	
Name	C.I. No.	Resistant	Susceptible
Bolivia	1257	1, 2, 4, 5, 11, 16, 35, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52.	3, 32, 34, 36.
Bongie	2479	2, 4, 37, 47, 49, 51, 52.	44, 45.
Brandon 1136 X Kindred	9553	4, 35, 37, 40, 44, 47, 49.	L-13, a subordinate of UN-4; and L-21, a subordinate of UN-44.
Brandon Selection Minn I-48-5	9187	4, 35, 37, 40, 44, 47, 49.	
Burgarab	3661	4, 37, 44, 45, 47, 49, 51, 52.	
California Mariout	1455	37; also C-9, a subordinate of UN-4.	1, 4, 5
Cape	557	4, 37.	1, 5.
Carre 26	3386	1, 4, 38, 39, 40, 51.	37, 44, 45, 47, 49, 52; also L-5, a subordinate of UN-4.
Carre 180	3390	2, 4, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	
Caspian	5644	2, 4, 38, 39, 40.	
Caucasus	4334	47, 49, 51, 52.	1, 2, 4, 11, 37, 44, 45.
Cebada Capa	6193	2, 4, 38, 39, 40.	
Chevalier	278	4, 37.	1, 5.
Chile Brewing	657	37; also C-9, a subordinate race of UN-4.	1, 4, 5.
Chilean D	1433	1, 2, 4, 11, 37, 38, 39, 40, 44, 46, 47, 49, 51.	3, 5, 16, 32, 34, 35, 36, 43, 45, 48, 50, 52; also B-4 and L-13, both subordinate races of UN-4.
Club Mariout	261	4, 5, 37, 38, 39, 40, 43, 44, 45, 48, 49; also C-13, a subordinate of UN-2.	1, 2, 3, 16, 32, 34, 35, 36, 47, 51, 52; also C-9 and L-18, both subordinates of UN-4; C-22, a subordinate of UN-5; and L-21, a subordinate of UN-44.
Coast	276	2, 4, 37, 38, 39, 40, 45, 47, 49.	44, 51, 52.
Coast	690	37; also C-9, a subordinate race of UN-4.	4.
Coast	1430	2, 5, 37, 38, 39, 40, 46, 48; also C-9, C-16, and L-7, all subordinate races of UN-4.	1, 3, 4, 11, 16, 32, 34, 35, 36, 43, 44, 45, 47, 50; also C-22, a subordinate race of UN-5.
Colsees	2792	4, 5, 34, 35, 37, 38, 39, 40, 43, 44, 48, 49.	1, 2, 3, 36.
Composite Cross VII Selections: 173, 175	6622	1, 4, 37, 44, 47, 49.	35, 40, 45.
Composite Cross VIII Selections: 177	6623	1, 4, 35, 37, 40, 44, 45, 47, 49.	
Composite Cross IX Selections: 179	6624	1, 4, 35, 37, 40, 44, 45, 47, 49	
Composite Cross XI Selections: 104, 109, 110, 117, 120, 121, 131, 138, 139, 184, 185 141, 142	6724	1, 4, 35, 37, 40, 44, 45, 47, 49 4, 37, 44, 47, 49.	1, 35, 40, 45; also L-18, a subordinate race of UN-4; and L-22, a subordinate race of UN-47

TABLE 1.—COMPARATIVE SEEDLING REACTION OF DIFFERENT VARIETIES AND SELECTIONS OF BARLEY TO VARIOUS CONSOLIDATED PHYSIOLOGIC RACES OF LEAF RUST—*Continued*

Varieties concerned		Reaction to specified races	
Name	C.I. No.	Resistant	Susceptible
Composite Cross XIII Selections: 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 154, 155, 156, 157, 158, 159, 160, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 183, 186, 189	7117	1, 4, 35, 37, 40, 44, 45, 47, 49	
153		4, 37, 44, 47, 49.	1, 35, 40, 45; also L-18, a subordinate race of UN-4; and L-22, a subordinate race of UN-47.
187, 188, 190		1, 35, 40, 45; also L-18, a subordinate of UN-4; and L-22, a subordinate race of UN-47.	4, 37, 44, 47, 49.
Crusat	6482	1, 2, 4, 11, 37, 38, 44, 46, 47, 49, 51, 52.	3, 5, 16, 32, 34, 35, 36, 39, 40, 43, 45, 48, 50; also L-13, a subordinate of UN-4.
Decorticatum	2230	4.	37; also C-9, a subordinate race of UN-4.
Deficiens	2225	4, 37.	C-9, a subordinate race of UN-4.
Dinar	729	1, 2, 4, 16, 37, 44, 47, 49, 51, 52.	40, 45.
Ey. Black Turkestan	3093	44.	2, 4, 37, 45, 47, 49, 51, 52.
Egypt 4	6481	37, 38, 39; also C-12, a subordinate of UN-2, and B-1, a subordinate of UN-4.	1, 2, 3, 4, 5, 11, 16, 32, 34, 35, 36, 40, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52.
Featherston	1120	1, 4, 5.	
Flynn	1311	2, 5, 37, 38, 39, 46, 48; also B-4, C-9, and C-16, all subordinates of UN-4; as well as L-7, also a subordinate of UN-4.	1, 3, 4, 11, 16, 32, 34, 35, 36, 40, 43, 44, 45, 47, 49, 50; also C-22, a subordinate race of UN-5.
Foreign 82B, CA-62		C-9, a subordinate race of UN-4.	4, 37.
French Chevalier	175	4, 37.	
Ghest	979	4, 45, 47.	2, 37, 44, 49, 51, 52.
Glabron	4577	1, 4, 5, 34, 35, 37.	3, 36.
Gold	1145	2, 4, 11, 32, 34, 36, 37, 39, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52.	1, 3, 5, 16, 35, 38, 40.
Grossklappige	6485	2; also C-9, C-16, and L-7, all subordinate races of UN-4.	1, 3, 4, 5, 11, 16, 32, 34, 35, 36, 37, 38, 39, 40, 43, 44, 45, 46, 47, 48, 50.
Gujrat	3397	2, 4, 37, 44, 45, 47, 49, 51, 52.	
Halikonohra	6004	4, 37.	
Hannchen	531	37.	1, 4, 5.
Harbine	7524	4, 44, 47, 52.	37, 45, 49, 51.
Heil Hanna 3	682	4, 37.	1, 5.
Hero	1286	4, 37.	C-9, a subordinate race of UN-4.
Hey Special	6487	1, 4, 11, 16, 43, 44, 45, 48.	2, 3, 5, 32, 46, 47, 49, 50.
Hietpas 5	7124	L-18, a subordinate race of UN-4.	3, 4, 5, 44, 47, 49.
Hokudo	5176	47; also L-18, a subordinate of UN-4.	1, 2, 4, 11, 16, 37, 40, 44, 45, 49, 51, 52; also L-22, a subordinate of UN-47.
Horsford	877	1, 4, 5, 16, 34, 35, 37, 38, 39, 40, 44, 45, 47, 48, 49.	2, 3, 11, 32, 36, 43, 46, 50.

TABLE 1.—COMPARATIVE SEEDLING REACTION OF DIFFERENT VARIETIES AND SELECTIONS OF BARLEY TO VARIOUS CONSOLIDATED PHYSIOLOGIC RACES OF LEAF RUST—*Continued*

Varieties concerned		Reaction to specified races	
Name	C.I. No.	Resistant	Susceptible
Hoshiarpur	3396	1, 2, 4, 11, 37, 44, 45, 47, 49, 51, 52.	
Kindred	6969	2, 40; also B-1, B-4, and C-16, all subordinate races of UN-4.	1, 4, 11, 37, 38, 39, 44, 45, 47.
Kinver	2361	W-2, a subordinate race of UN-3	1, 3, 4, 37, 44.
Kuban	6480	1, 2, 4, 11, 16, 32, 37, 38, 39, 40, 43, 44, 46, 47, 48, 49, 50, 51, 52.	3, 5, 34, 35, 36, 45.
Kura	4306	47; also L-18, a subordinate of UN-4, L-21, a subordinate of UN-44, and L-23, a subordinate of UN-45.	1, 4, 11, 37, 44, 45, 49, 51, 52.
Kwan	1016	1, 2, 4, 11, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	
Lechtaler	6488	1, 2, 4, 11, 32, 37, 43, 44, 46, 47, 48, 49.	3, 5, 16, 40, 45, 50, 51, 52.
Lico	6279	4.	37.
Luth	972	1, 4, 11, 37, 38, 39, 40, 43, 44, 45, 47, 48, 49, 50, 51, 52; also C-12, a subordinate of UN-2.	2, 3, 5, 16.
Lyallpur BS	3395	2, 4, 37, 44, 45, 47, 49, 51, 52.	
Manchurian	739	4, 37.	
Manchuria	2330	1, 4, 44, 45, 47.	11, 43, 46, 50.
Marco	5647	2, 4, 38, 39, 40.	
Menelik	5862	2, 4, 38, 39, 40.	
Mensury	4696	4, 37.	
Mianwali	3400	2, 4, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	
Michigan 2-row	2782	4, 37.	
Modia	2483	1, 2, 4, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	L-18, L-21, and L-23, respective subordinate races of UN-4, UN-44, and UN-45.
Montcalm	7149	4, 40.	2, 38, 39; also B-4 and C-9, both UN-4 subordinates.
Morocco	3902-1	1, 2, 4, 11, 38, 39, 40, 44, 45.	
Morocco	6311	1, 2, 4, 11, 38, 39, 40, 44, 45.	
Mortoni	2210	B-1, a subordinate race of UN-4.	1, 4, 11, 37, 44, 45.
Nameless	2349	4, 51.	2, 37, 44, 45, 47, 49, 52.
Forjara	2538	2, 4; 38, 40, 45, 47, 51.	37, 39, 44, 49, 52; also C-13, a subordinate race of UN-2.
Bari	2542	2, 4, 38, 39.	37, 40, 44, 45, 47, 49, 51, 52.
Nameless	2549	2, 4, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	
Nameless	3356	4.	2, 37, 44, 45, 47, 49, 51, 52.
Nameless	3373	1, 4, 11, 44, 45.	
Nameless	3737	2, 4, 38, 39, 40.	
Nameless	4219	2, 4, 38, 39, 40.	
Nameless	4220-1	2, 4, 38, 39, 40.	
Nameless	4223-3	2, 4, 38, 39, 40.	

TABLE 1.—COMPARATIVE SEEDLING REACTION OF DIFFERENT VARIETIES AND SELECTIONS OF BARLEY TO VARIOUS CONSOLIDATED PHYSIOLOGIC RACES OF LEAF RUST—*Continued*

Varieties concerned		Reaction to specified races	
Name	C.I. No.	Resistant	Susceptible
Nameless	4230-1	1, 4, 44, 45.	11.
Nameless	4290	4.	2, 37, 44, 45, 47, 49, 51, 52.
Nameless	4295	51, 52; also L-18, and L-21, subordinates of UN-4, and UN-44, respectively.	1, 2, 4, 11, 37, 44, 45, 47, 49.
Nameless	4298	51, 52; also L-18, L-21, and L-23, subordinates of UN-4, UN-44, and UN-45, respectively.	1, 2, 4, 11, 37, 44, 45, 47, 49.
Nameless	4308-2	2, 4, 37, 44, 45, 47, 49, 51, 52.	
Nameless	4309	44, 45, 51, 52.	2, 4, 37, 47, 49.
Nameless	4310	4.	2, 37, 44, 45, 47, 49, 51, 52.
Nameless	4314	4, 47, 49, 51.	2, 37, 44, 45, 52.
Nameless	4356	2, 4, 38, 39.	40.
Nameless	4473	37, 44, 45, 47, 49, 52.	4, 51.
Nameless	4573-1	4, 37, 44, 45, 47, 49, 51, 52.	
Nameless	4972	47, 49.	2, 4, 37, 44, 45, 51, 52.
Nameless	4974	2, 4, 38, 39, 40.	
Nameless	4975	1, 2, 4, 11, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	
Nameless	5326	4, 38, 39.	2, 40.
Nameless	5366	2, 4, 38, 39, 40.	
Nameless	5564	2, 4, 37, 44, 45, 47, 49, 51, 52.	
Nameless	5863	2, 4, 38, 39, 40.	
Nepal	595	5, 37; also B-4, a subordinate race of UN-4.	1, 3, 4, 34, 35, 36; also C-23, a subordinate race of UN-37.
O. A. C. 21	1470	1, 4, 16, 34, 35, 37, 38, 39, 40, 43, 44, 47, 48, 49; also C-13, a subordinate race of UN-2.	2, 3, 5, 36; also B-4, a subordinate race of UN-4.
O. A. C. 620	4872	4, 37.	
Oderbrucker	940	1, 4, 5, 16, 34, 35, 37, 38, 39, 40, 44, 45, 47, 48, 49, 51, 52.	2, 3, 11, 32, 36, 43, 46, 50.
Palmella Blue	3609	2, 4, 38, 39, 40.	
Pannier	1330	4, 37.	
Paso	5047	2, 4, 38, 39, 40.	
Peruvian	935	1, 4, 5.	37.
Peruvian	1131	5, 37; also C-9, a subordinate race of UN-4.	1, 4.
Peruvian 1	5912	2, 4, 11, 32, 38, 39, 40, 43, 44, 45, 47, 48, 49.	1, 3, 5, 16; also L-21, a subordinate race of UN-44.
Peruvian 19	6568	2, 4, 38, 39, 40.	
Psaknon	6305	B-1, a subordinate race of UN-4; and C-12, a subordinate of UN-2.	2, 4, 38, 39, 40.
Purple Nepal	2242	2, 4, 38, 39, 40.	
Quinn	1024	1, 2, 3, 4, 11, 16, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52.	5.

TABLE 1.—COMPARATIVE SEEDLING REACTION OF DIFFERENT VARIETIES AND SELECTIONS OF BARLEY TO VARIOUS CONSOLIDATED PHYSIOLOGIC RACES OF LEAF RUST—*Concluded*

Varieties concerned		Reaction to specified races	
Name	C.I. No.	Resistant	Susceptible
Rabat	4979	1, 2, 4, 16, 37, 38, 39, 40, 44, 45, 47, 49, 51, 52.	
Reka 1	5051	1, 2, 4, 11, 32, 37, 38, 39, 40, 43, 45, 46, 47, 48, 50, 51.	3, 5, 16, 34, 35, 36, 44, 49, 52; also C-13, a subordinate race of UN-2.
Ricardo	6306	2, 4, 38, 39, 40.	
Rimpaui	2220	4, 37, 38, 39, 40.	2.
Rowan	5672	1, 4, 44, 45.	11.
Sacramento	4108	4, 37.	
Schladener	6490	1, 4, 11, 16, 43, 44, 45, 48.	3, 5, 46, 47, 50.
Spartan	5027	46.	1, 4, 11, 43, 44, 45, 47.
Speciale	7536	1, 4, 11, 16, 37, 40, 43, 44, 45, 48.	2, 3, 5, 32, 46, 47, 49, 50, 51, 52.
Speciale A	7536-1	4, 37, 44, 45.	51.
Speciale B	7536-2	4, 37, 44, 45.	51.
Stavropol	2103	1, 4, 5.	B-4, a subordinate race of UN-4.
Steudelii	2226	4, 37.	
Success	4840	1, 4, 5, 37.	
Sudan	6489	1, 3, 4, 5, 16, 34, 35, 36, 37, 38, 40, 43, 44, 45, 46, 47, 49, 50, 52.	2, 11, 32, 39, 41, 42, 48, 51.
Sulu	1022	2, 4, 11, 43, 44, 45, 46, 47, 48, 49.	1, 3, 5, 16.
Telli	194	4, 37, 44, 45, 47, 49, 51, 52.	
Tenarab	662	2, 4, 37, 44, 45, 47, 49, 51, 52.	
Tennessee Winter	257	5.	1, 4, 37.
Tricerros	2227	4.	37; also C-9, a subordinate race of UN-4.
Tridax	2228	4.	37.
Velvet	4252	46.	1, 4, 5, 11, 37, 43, 44, 45, 47.
Villa	3527-2	4, 37, 44, 45, 47, 49, 51, 52.	
Weider	1021	1, 2, 3, 4, 5, 11, 16, 32, 37, 40, 43, 44, 45, 46, 47, 48, 49, 50, 51.	
Bolron	7123	1, 2, 4, 16, 37, 40, 44, 47, 49.	

List of Susceptible Varieties.—(Varietal name is followed by C.I. number; in parentheses are listed races to which the variety was tested.)—*Angustispicatum* 2219 (4, 37). *Arlington Awnless* 702 (4, 37, 44, 45, 47, 49, 51, 52), *Atlas* 4118 (1, 4, 11, 37, 44, 45), *Atrum* 2204 (4, 37), *Baku* 709 (1, 4, 5, 37), *Barbless* 5105 (1, 4, 11, 37, 43, 44, 45, 46, 47), *Bethges III* 6484 (1, 4, 11, 43, 44, 45, 46, 47), *Black Hulless* 666 (1, 4, 5, 37), *Byng* 6089 (4, 37), *California Brewing* 4870 (4, 37), *Canadian Thorpe* 740 (1, 4, 5, 37), *Chevron* 1111 (1, 2, 4, 5, 11, 16, 35, 37, 40, 43, 44, 45, 46, 47, 49, 51, 52), *Composite Cross VII* 6622 sel. 174 (1, 4, 35, 37, 40, 44, 45, 47, 49), *Composite Cross VIII* 6623 sels. 176, 178 (1, 4, 35, 37, 40, 44, 45, 47, 49), *Composite Cross IX* 6624 sel. 180 (1, 4, 35, 37, 40, 44, 45, 47, 49), *Composite Cross XI* 6724 sels. 101, 102, 103, 105, 106, 107, 108, 111, 112, 113, 114, 115, 116, 118, 119, 122, 123, 124, 125, 126, 127, 128, 129, 130, 132, 133, 134, 135, 136, 137, 140, 181 (1, 4, 35, 37, 40, 44, 45, 47, 49), *Composite Cross XIII* 7117 sels. 161, 182 (1, 4, 35, 37, 40, 44, 45, 47, 49), *Cornutum* 2215 (4, 37), *Danubian* 6525 (4, 37), *Dorsett* 4821 (1, 4, 11, 44, 45), *Duab* 4147-2 (4, 37, 44, 45, 47, 49, 51, 52), *Duckbill* 1916 (4, 37), *Duplex* 2433 (2, 4, 5, 37, 44, 45, 47, 49, 51, 52), *Franconian* 680 (1, 4, 5, 37), *Garton* 645 (4, 37), *Golden Drop* 2135 (4, 37), *Goldfoil* 928 (1, 3, 4, 37, 44, 45, 47, 49, 51, 52), *Gordon* 4842 (4, 37), *Greece* 221 (2, 4, 37, 44, 45, 47, 49, 51, 52), *Hanna* 203 (1, 4, 5, 37), *Hanna* 906 (1, 4, 11, 37, 43, 44, 45, 46, 47, 49, 51, 52), *Hanna* 1122 (1, 4, 5, 37), *Italian* 914 (4, 37), *Jet* 2222 (4, 37), *Kindred* x *Titan*, N.D. B103 9538 (4, 35, 40, 44, 47, 49), *Kite* 992 (1, 4, 11, 43, 44, 45, 46, 47), *Kozan* 5172 (4, 37, 44, 45, 47, 49, 51, 52), *Lion* 923 (4, 37), *Malting* 1129 (4), *Marm* 5562 (1, 4, 11, 44, 45), *Minn.* 4228 (4, 37, 44, 45, 47, 49, 51, 52), *Minn.* II-31-19 7011 (1, 4, 11, 44, 45, 50), *Minn.* II-41-2 7560 (2, 4, 37, 44, 45, 47, 49, 51, 52), *Minsturdy* 1556 (1, 4, 11, 37, 43, 44, 45, 46, 47), *Mo.* B-576 8079 (4, 37, 44, 45, 47, 49, 51, 52), *Moore* 7251 (1, 2, 4, 16, 37, 40, 44, 45, 47, 49, 51, 52), *Nameless* 3916-4 (4, 37, 44, 45, 47, 49, 51, 52), *Nameless* 3967 (1, 4, 11, 44, 45), *Nameless* 4160-1 (2, 4, 38, 39, 40), *Nameless* 4326-1 (1, 4, 11, 44, 45), *Newal* 6088 (4, 37), *Newal-Peatland* x *Montcalm*, U.M.-570 9545 (4, 35, 40, 44, 47, 49), *Nigrilaxum* 2224 (4, 37), *Nudihaxtoni* 2213 (4, 37), *Odessa* 182 (1, 4, 5, 37), *Olli* 6251 (4, 37), *Orel* 351 (1, 4, 5, 37), *Pearl* 4834 (4, 37), *Peatland* 5267 (1, 3, 4, 5, 11, 34, 35, 36, 37, 43, 44, 45, 46, 47), *Peking* 4202-2 (1, 4, 11, 44, 45), *Pliter* 6036 (1, 4, 11, 43, 44, 45, 46, 47), *Plumage Archer* 5033

List of Susceptible Varieties—Concluded.

(4, 37), Plush 7030 (4, 37), Princess 529 (4, 5, 37), Regal 5030 (4, 37), Riegel 7328 (2, 4, 5, 37, 44, 47, 49, 50, 52), Samaria 6493 (1, 4, 11, 43, 44, 45, 46, 47), Shimabara 5196 (4, 37, 44, 45, 47, 49, 51, 52), Silver King 890 (4, 37), Spiti 4343-1 (4, 37, 44, 45, 47, 49, 51, 52), Stella 2678 (4, 37), Stephan 8051 (4, 37), Suchow 5091 (1, 4, 11, 44, 45), Svansota 1907 (1, 4, 11, 43, 44, 45, 46, 47), Swedish Star 1701 (4, 37), Taxan 6499 (4, 37), Titan 7055 (2, 4, 38, 39, 40), Tradak 5645 (4, 37, 44, 45, 47, 49, 51, 52), Trebi 936 (1, 4, 5, 11, 37, 44, 45), Tregal 6359 (2, 4, 38, 39, 40), Trifurcatum 2207 (4, 37), Vagabond 3933 (4, 37, 44, 45, 47, 49, 51, 52), Vaughan 1367 (4, 37), Velvon 6109 (2, 4, 38, 39, 40), Warrior 6991 (2, 4, 38, 39, 40), White Gatami 920 (2, 4, 38, 39, 40).

The study of the varietal reaction of adult barley plants to leaf rust under natural field infections was complementary to that of seedling reaction under controlled greenhouse conditions, even though the two studies were not entirely concurrent chronologically. In the course of the 20-year period, 1935-1954, an even 100 varieties and selections of barley were grown in a varying number of uniform nurseries maintained in different parts of the United States. Only one of these, Velvet (C.I. 4252), had been grown each of the 20 years concerned; two other varieties, Bolivia (C.I. 1257) and Chevron (C.I. 1111), were grown for 17 years; the remaining varieties and selections were grown for shorter periods, down to one year for some of them. The severity of the leaf rust epidemics varied from nursery to nursery and from year to year; and no leaf rust whatever could be found in the barley growing region during 1936 and 1937, either in the uniform nurseries or in farmers' fields.

Nearly half of the varieties tested in the uniform rust observation nurseries had also been tested in the seedling stage. The severity of leaf rust infection in the nurseries was recorded in percentage terms, based on the so-called modified Cobb scale generally used by cereal rust workers, and then converted into infection coefficients.* The results obtained in the nurseries most severely affected in any given year are recorded in Table 2. The most intense average leaf rust infection during any one of the 20 years under consideration, exclusive of 1936 and 1937 when no leaf rust whatever could be found, occurred in the following nurseries: 1935—Waseca, Minn. (26.3%); 1938—Ames, Iowa (34.0%); 1939—East Lansing, Mich. (58.0%); 1940—East Lansing (46.2%); 1941—Kanawha, Iowa (56.7%); 1942—Lafayette, Ind. (69.8%); 1943—Madison, Wis. (25.5%); 1944—Madison (25.8%); 1945—Manhattan, Kans. (45.0%); 1946—Lafayette (53.8%); 1947—East Lansing (17.2%); 1948—Lafayette (22.8%); 1949—Raleigh, N.C. (26.0%); 1950—Lafayette (58.3%); 1951—Urbana, Ill. (42.9%); 1952—Ames (8.3%); 1953—Ithaca, N.Y. (30.3%); and 1954—Ithaca (42.7%).

An examination of the data recorded in Table 2 will reveal that, out of the 100 varieties and selections tested in the uniform barley nurseries, only the following three were consistently resistant: Lyallpur E (C.I. 3403), Rabat (C.I. 4979), and C.I. 7122. At no time, nor at any place, had any of these barleys scored infection coefficients in excess of 3 per cent. Varieties Halikonohra (C.I. 6004), Sacramento (C.I. 4108), and Servian (C.I. 915) were tested during a period of 1935-1937 when during two of these years, 1936 and 1937, no leaf rust occurred. Therefore, data on their resistance cannot be considered conclusive.

Slightly more susceptible were eight additional varieties, whose infection coefficients were never higher than 8 per cent at any given nursery,

* Infection coefficient is determined by multiplying the estimated rust percentages by the numerical values assigned to the different degrees of host response. These latter are: completely susceptible = 1.0, susceptible = 0.8, intermediate = 0.6, resistant = 0.4, highly resistant = 0.2, and immune = 0.

and their averages in the nursery territory as a whole were considerably lower. These varieties were: Black Hulless (C.I. 596), Estate (C.I. 3410), Gust Plantz 12 (C.I. 7173), Kurof (C.I. 1098), Nepal (C.I. 595), Oderbrucker (C.I. 4666), Princess (C.I. 529), and Bolron (C.I. 7123). Of these, only Bolron had been tested in the uniform nurseries for as many as 11 years; Estate had been tested for 8 years; Oderbrucker for 3 years; Black Hulless, Kurof, Nepal, and Princess for 2 years; Gust Plantz 12 for 1 year.

Perhaps even more significant than the records of the foregoing reactions is the fact that certain barley varieties and selections, having the reputation of high resistance over a long period of time and under a great variety of conditions, occasionally do succumb to severe dwarf leaf rust epidemics. This in itself may not necessarily be a cause for alarm, except that similar experiences with other crops and other rusts should caution against excessive optimism and suggest instead continuous vigilance. Not infrequently, danger-signs like those discussed hereunder have in the past been precursors of immediate or eventual "breakdowns" of varieties which had long been reputed for their outstanding resistance.

In the course of the past 20 years, the following seven varieties had good records of average leaf rust resistance in the uniform nursery tests. There were, however, in each case also notable exceptions. The varieties discussed hereunder had been tested for from 10 to 17 years and at a minimum of 6 and a maximum of 16 nurseries in any given year.

Abyssinian (C.I. 1243), which had been grown during the 14-year period 1941-1954, had been subjected to a total of 190 nursery tests. Its infection coefficient during this period averaged 1.7 per cent. However, in 1941 it scored an infection coefficient of 60 per cent at Kanawha, Iowa, as well as at Urbana, Ill.

Bolivia (C.I. 1257), which has had a total of 222 nursery tests during the 17-year period of 1938-1954, averaged 1.6 per cent, but attained a maximum infection coefficient of 72 per cent at Urbana in 1951.

Chilean D (C.I. 1433) has had an over-all infection average of 1.3 per cent for a total of 218 nursery tests during the same 17 years as above. Its maximum infection of 45 per cent was attained in 1940 at Waseca, Minn.

Luth (C.I. 972), averaged 1.2 per cent over the 10-year period 1945-1954, during which it had been subjected to a total of 137 nursery tests. It scored a high of 75 per cent in 1951 at Urbana.

Morocco (C.I. 6311) had scored the lowest average of 0.8 per cent for the 163 nursery tests it had during the 12-year period of 1943-1954. Its highest infection coefficient at any time was 90 per cent in 1951 at Urbana.

Nameless (C.I. 4975) reached a high of 100 per cent in 1940 at Urbana, although its over-all infection average for 133 nursery tests over the 10-year period of 1943-1952 was only 1.2 per cent.

Quinn (C.I. 1024), which had been subjected to a total of 214 nursery tests over the 16-year period of 1939-1954, has had an over-all average of 1.1 per cent. Its maximum infection coefficient of 60 per cent occurred at East Lansing, Mich., in 1940.

TABLE 2.—RELATIVE SUSCEPTIBILITY OF CERTAIN VARIETIES AND SELECTIONS OF BARLEY TO NATURALLY OCCURRING DWARF LEAF RUST EPIDEMICS IN THE MOST SEVERELY AFFECTED CO-OPERATIVE UNIFORM OBSERVATION NURSERIES, GROWN IN VARIOUS PARTS OF THE UNITED STATES. DATA ARE FOR 1935, AND 1938 TO 1954.

Varieties tested		Coefficient* of infection during specified years																	
Name	C.I. No.**	1935	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954
Abyssinian	1243	—	—	—	—	60	6	T	T	1	0	4	T	T	T	60	8	4	4
Afghan 2	6366	—	—	—	—	63	—	—	—	15	60	10	68	—	—	—	—	—	—
Atlas	4118	2	40	8	15	90	100	40	70	80	40	24	58	—	—	—	—	—	—
Barbless	5105	13	24	85	—	—	—	—	—	100	100	—	—	—	—	—	—	—	—
Bay	7113	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Black Hulless	596	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Black Hulless	666	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bolivia	1257	—	1	3	0	T	T	T	T	T	0	—	0	—	—	72	6	—	85
Bon Ruden 2	6606	—	—	—	—	—	30	7	12	—	—	—	—	—	—	—	—	—	T
Brandon 1136	9535	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Brandon Selection, Minn. I-48-5	9187	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CC 6724-109	8089	—	—	—	—	95	100	63	81	80	100	52	68	40	75	56	12	45	50
Chevron	1111	—	86	60	75	3	6	T	T	T	T	T	3	9	4	100	T	—	75
Chilean D	1433	—	3	6	4	—	—	—	—	—	—	—	—	—	0	0	—	25	12
Colsees	2792	15	—	—	—	—	—	—	—	—	—	—	—	—	—	100	6	—	75
Comfort	4578	48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Estate	3410	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ezond	6265	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glaron	4577	21	65	90	20	90	—	—	—	—	—	—	—	—	—	—	—	—	—
Goliad	8099	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gospick	9094	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gust Plautz 12	7173	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
H-111-87	9185	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Halkonohra	6004	1	36	80	85	80	100	70	40	80	90	36	20	60	80	0	8	5	0
Hanna	906	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Harlan	7008	39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Heinrich	6354	—	5	—	—	—	—	36	56	—	—	—	—	—	—	—	—	—	—
Hietpas 3	6611	—	—	—	—	—	100	16	54	15	40	3	2	25	90	4	T	30	35
Hietpas 5	7124	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Himalaya	620	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiraud	6355	—	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
India Hulless	698	—	100	95	80	90	—	54	42	42	100	15	40	30	60	T	12	75	75
Kindred	6969	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85
Kindred X Titan, N.D. B103	9538	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kite	992	15	2	75	60	75	100	—	—	—	—	—	—	—	—	—	—	—	—
Kurof	1098	5	63	85	85	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kusan	1315	—	40	40	7	10	100	—	—	—	—	—	—	—	—	—	—	—	—
Leh	700	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lion	923	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Luth	972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lyallpur E	3403	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Manchuria	2330	52	20	75	4	15	T	9	T	3	0	1	0	T	1	75	6	2	1
Manchuria	2947	63	45	15	65	85	100	27	21	24	60	24	32	—	—	—	—	45	—
Mariotti	6356	—	18	3	50	49	—	—	—	—	—	—	—	—	—	—	—	—	—
Mars	7015	—	—	—	—	—	—	—	—	90	100	—	—	—	—	—	—	—	—
Mars	7141	—	—	—	—	—	—	—	—	80	72	32	3	35	95	80	T	85	—
Minn. II-37-15	7560	—	—	—	—	—	—	—	—	—	—	—	—	65	75	—	2	35	80

Data on the reaction of mature plants of the 234 barley varieties and selections that were tested at the Winnipeg nursery during 1944-1947, inclusive, are presented in Table 3. Nearly two-thirds of these barleys never exceeded a leaf rust infection average of 2.5 per cent. Included among these were such varieties as Rabat (C.I. 4979) and Sacramento (C.I. 4108), which in the uniform rust observation nurseries had no infection percentages of this magnitude at any time. The variety Nepal (C.I. 595), whose maximum infection coefficient in the uniform nurseries was 8 per cent, attained one 2-year average of 30 per cent in the Winnipeg nursery. Conversely, several other varieties, which never averaged more than 15 per cent of leaf rust during a given biennium in the Winnipeg nursery, on occasion averaged as much as 85 per cent during a preceding or following biennium. Such varieties as Archer Goldthorpe (C.A. 1003), Austrian Hanna St. 66 (C.A. 46), Baker (C.A. 87), German Brewing (C.A. 1008), Golden Drop (C.A. 49), Plumage Archer (C.A. 1004), and Success (C.A. 783), illustrate this point. Different racial composition of the inoculum in different years might have been responsible for the fluctuation in the amount of infection.

Whereas at least 3 of the varieties tested at Winnipeg—Glacier (C.I. 6976), Steigum (C.I. 907), and Svansota (C.I. 1907)—had 2- or 4-year weighted averages of as high as 90 per cent, the following 3 varieties had been entirely free of leaf rust during the 4 years of the test: Batna (C.I. 3391), Bolivia (C.I. 1257), and Rabat (C.I. 4979). Among the 36 barleys that had no more than a trace of leaf rust at any time in the Winnipeg nursery, there were 21 that were resistant in the seedling stage to whatever races they were tested with. These were: Abacus (C.I. 1088), Barley 305 (C.I. 6015), Batna (C.I. 3391), Carre 180 (C.I. 3390), Caspian (C.I. 5644), Cebada Capa (C.I. 6193), Kwan (C.I. 1016), Marco (C.I. 5647), Morocco (C.I. 3902-1), Morocco (C.I. 6311), Peruvian 19 (C.I. 6568), Purple Nepal (C.I. 2242), Rabat (C.I. 4979), Ricardo (C.I. 6306), Sacramento (C.I. 4108), Telli (C.I. 194), and the 5 nameless varieties, C.I. 2549, C.I. 3737, C.I. 4223-3, C. I. 4974, and C.I. 4975.

DISCUSSION AND CONCLUSIONS

Data summarized in this paper indicate the existence of barley varieties highly resistant or immune to prevalent races of leaf rust. It should be possible, therefore, to transfer such resistance to varieties of barley agronomically suitable in regions where leaf rust epidemics occur frequently.

The rather frequent mutation in this rust (4) and the occurrence of a number of variants, "subordinate races", indicate the presence in the leaf rust fungus of numerous biotypes which makes the development of resistant varieties more difficult. However, barley varieties showing resistance consistently in both the seedling and adult plant stages may contain genes for resistance to which the pathogen cannot readily adapt itself.

By and large, the results of field plot tests were in agreement with those on seedling reactions obtained in greenhouse tests, except for an occasional variety deviating towards greater resistance in the adult plant stage. There were other deviations, however, both in the Winnipeg nursery under artificially induced epidemics, and in the United States

TABLE 3.—RELATIVE SUSCEPTIBILITY OF CERTAIN VARIETIES AND SELECTIONS OF BARLEY GROWN IN FIELD PLOTS UNDER ARTIFICIALLY INDUCED EPIDEMICS OF DWARF LEAF RUST AT WINNIPEG, MANITOBA, DURING THE 4-YEAR PERIOD 1944-1947, INCLUSIVE

Varieties tested		Av. infection		Varieties tested		Av. infection	
Name	C.I. No.*	1944-45	1946-47	Name	C.I. No.	1944-45	1946-47
Abacus	1088	0.2	0.0	Glacier	6976	—	90.0
Abyssinian	2192	0.2	7.5	Gold	1145	0.2	1.5
Aethiops	2208	17.5	10.0	Golden Drop	2135	12.5	60.0
Afghan 2	6366	5.0	16.0	Golden Pheasant	2488	—	75.0
Algerian	1179	0.0	1.0	Goldfoil	928	27.5	75.0
Alpha	959	70.0	40.0	Gordon	4842	75.0	87.5
Angustispicatum	2219	87.5	32.5	Grossklappige	6485	—	50.0
Apalan	1347	—	50.0	Halikon	—	0.2	1.0
Archer	1031	—	80.0	Halikonohra	6004	10.0	7.5
Archer Goldthorpe	—	5.0	70.0	Hanna	203	42.5	30.0
Arequipa	2329	0.2	2.5	Hanna	906	37.5	50.0
Arlington Awnless	702	—	77.5	Hanna	1122	47.5	70.0
Atlas	4118	45.0	67.5	Hannchen	531	50.0	62.5
Atrum	2204	27.5	17.5	Heil Hanna 3	682	0.2	0.2
Austral	6483	1.0	2.5	Hero	1286	40.0	27.5
Austrian Hanna St. 66	—	7.5	50.0	Hero	4602	—	70.0
Baker	975	15.0	85.0	Himalaya	620	60.0	42.5
Baku	709	—	55.0	Hooded Spring	716	—	42.5
Barbless	5105	50.0	57.5	Horn	926	40.0	82.5
Barboff	7148	—	20.0	Horsford	507	35.0	20.0
Bark	2793	47.5	60.0	Horsford	1775	55.0	82.5
Barley 305	6015	0.2	0.2	Icelandic	—	85.0	72.5
Batna	3391	0.0	0.0	Italian	914	5.0	10.0
Bavaria	6395	1.0	15.0	Jet	2222	67.5	60.0
Beecher	6566	62.5	70.0	Julia	1114	—	0.2
Beldi Dwarf	190	—	35.0	Kindred	6969	47.5	27.5
Beldi Giant	2777	0.2	10.0	Korsbyg	918	—	4.0
Berg	6486	1.0	1.0	Kuban	6480	0.2	1.0
Black Barbless	1993	25.0	80.0	Kwan	1016	0.2	0.0
Blackhall	878	47.5	77.5	Lennoxville	—	—	15.0
Black Hulless	666	22.5	25.0	Lico	6279	10.0	55.0
Blue Hulless	4848	42.5	45.0	Lion	—	20.0	60.0
Boehmes Beardless	2203	22.5	25.0	Lion	923	—	65.0
Bolivia	1257	0.0	0.0	Luth	972	0.2	0.2
Byng	6089	30.0	75.0	Malting	1129	—	30.0
California Brewing	4870	17.5	52.5	Manchuria	739	7.5	20.0
California Coast	6115	27.5	55.0	Manchuria	2330	17.5	10.0
California Mariout	1455	0.2	5.0	Mansfield	2241	80.0	85.0
Callas	2440	—	35.0	Marco	5647	0.2	0.0
Canadian Thorpe	740	55.0	75.0	Mariout B113	—	1.0	—
Cape	557	0.2	15.0	Mars	7015	—	77.5
Carre 26	3386	2.0	1.0	Macknes Maroc	1379	—	0.2
Carre 180	3390	0.2	0.0	Mensury	4696	17.5	20.0
Caspian	5644	0.2	0.2	Mianwali	3400	5.0	0.2
Cebada Capa	6193	0.0	0.2	Michigan 2-row	2782	5.0	55.0
Charlottetown 80	2732	55.0	70.0	Michigan 110	9533	—	65.0
Chevalier	278	0.2	5.0	Minsturdy	1556	55.0	77.5
Chevron	1111	70.0	67.5	Modia	2483	0.2	0.2
Chile Brewing	657	0.2	0.2	Montcalm	7149	52.5	55.0
Chilean D	1433	0.2	0.2	Morocco	3902-1	0.2	0.0
Chili	1654	—	0.2	Morocco	6311	0.2	0.2
Clifford	1910	—	80.0	Morocco 077	—	1.5	4.5
Club Mariout	261	0.2	0.2	Mortoni	2210	75.0	57.5
Coast	276	0.2	0.2	Nameless	510	—	50.0
Coast	1430	1.0	6.5	Forjara	2538	0.2	0.2
Coast	690	1.0	2.5	Bari	2542	0.2	0.2
Colsess	2792	17.5	17.0	Nameless	2549	0.0	0.2
Cornutum	2215	70.0	52.5	Nameless	3737	0.2	0.0
Cruzat	6482	0.2	1.0	Nameless	3770	—	5.0
Danish Island	—	45.0	75.0	Nameless	4160-1	17.5	22.5
Danubian	6525	7.5	25.0	Nameless	4223-3	0.2	0.2
Decorticatum	2230	52.5	77.5	Nameless	4356	17.5	12.5
Deficiens	2225	20.0	25.0	Nameless	4974	0.2	0.2
Dorsett	4821	—	60.0	Nameless	4975	0.2	0.2
Duckbill	1916	67.5	85.0	Nameless	5326	15.0	6.0
Duplex	2433	32.5	30.0	Nameless	5366	5.0	1.0
Egypt 4	6481	0.0	0.2	Nepal	595	10.0	30.0
Featherston	1120	20.0	17.5	Nepal	—	27.5	10.0
Flynn	1311	0.0	0.2	Newal	6088	75.0	70.0
Foreign 82B	—	1.5	17.5	Nigrilaxum	2224	67.5	47.5
Foreign 127	—	40.0	70.0	Nigrum	—	85.0	67.5
Franconian	680	10.0	45.0	Nobarb	6335	7.5	17.5
French Chevalier	175	0.2	4.5	Nobarb x Duplex	—	—	2.0
Galore	7150	—	45.0	Nudihaxtoni	2213	57.5	80.0
Garton 986	645	7.5	25.0	Nudimortoni	2214	57.5	67.5
Gatami	575	77.5	70.0	Nudum	—	32.5	22.5
Gatami	2276	—	40.0	O.A.C. 21	1470	37.5	40.0
German Brewing	—	12.5	70.0	O.A.C. 620	4872	1.0	1.0
Glaboron	4577	25.0	17.5				

*Cereal Introduction number, U.S.D.A.

TABLE 3.—RELATIVE SUSCEPTIBILITY OF CERTAIN VARIETIES AND SELECTIONS OF BARLEY GROWN IN FIELD PLOTS UNDER ARTIFICIALLY INDUCED EPIDEMICS OF DWARF LEAF RUST AT WINNIPEG, MANITOBA, DURING THE 4-YEAR PERIOD 1944-1947, INCLUSIVE—*Continued*

Varieties tested		Av. infection		Varieties tested		Av. infection	
Name	C.I. No.*	1944-45	1946-47	Name	C.I. No.	1944-45	1946-47
O.A.C. x Peatland	—	77.5	—	Sacramento	4108	0.2	0.2
U.A.M.	—	—	—	Sanalta	6087	—	60.0
O.C. x Peatland	—	45.0	—	Sandrel	937	72.5	77.5
U.M.	—	—	—	Scotch Standwell	—	40.0	72.5
Oderbrucker	940	17.5	40.0	Silver King	890	47.5	62.5
Oderbrucker	957	—	15.0	Smyrna	195	90.0	85.0
Oderbrucker	4666	—	5.0	Smyrna	—	50.0	60.0
Odessa	182	37.5	50.0	Spartan	5027	—	70.0
Olli	6251	87.5	80.0	Stavropol	2103	0.2	0.2
Orel	351	77.5	85.0	Steigum	907	90.0	90.0
Orge B100	—	0.0	0.2	Stella	2678	55.0	52.5
Orge 14B101	—	0.0	0.2	Stephan	8051	45.0	62.5
Palmella Blue	3609	1.5	2.5	Steudelii	2226	45.0	50.0
Pannier	1330	60.0	50.0	Success	4840	50.0	15.0
Paso	5047	0.0	1.0	Svalof	—	55.0	80.0
Pearl	4834	22.5	15.0	Svalof Victory	—	72.5	82.5
Peatland	5267	55.0	57.5	Svanhals	187	60.0	77.5
Persicum 064 (K)	6531	—	63.0	Svansota	1907	—	90.0
Peru	2302	65.0	45.0	Swedish Star	1701	80.0	90.0
Peruvian	935	0.2	0.2	Telli	194	—	0.0
Peruvian 1	5912	0.2	0.0	Texan	6499	70.0	67.5
Peruvian 19	6568	0.2	0.2	Titan	7055	87.5	72.5
Plumage Archer	5033	12.5	40.0	Trebi	936	65.0	75.0
Plush	7030	65.0	85.0	Tregal	6359	62.5	82.5
Polish	—	27.5	40.0	Triceros	2227	42.5	65.0
Pontiac	4849	45.0	55.0	Tridax	2228	60.0	75.0
Princess	529	10.0	12.5	Trifurcatum	2207	4.0	10.0
Prospect	6339	35.0	77.5	Vaughn	1367	50.0	45.0
Prussian	—	7.5	42.5	Velvet	4252	52.5	77.5
Psaknon	6305	1.0	2.0	Velvet-Olli x	—	—	42.5
Purple Nepal	2242	0.0	0.2	Peatland-Duplex	—	—	—
Quinn	1024	—	20.0	Velvet-Olli x	—	—	60.0
Rabat	4979	0.0	0.0	Peatland-Duplex	—	—	—
Regal	5030	65.0	85.0	Velvet-Olli x	—	—	35.0
Regal-Manchuria x	—	—	55.0	Peatland-Duplex	—	—	—
Peatland-Duplex	—	—	—	Velvon	6109	65.0	60.0
Regal-Manchuria x	—	—	65.0	Virginia Hooded	2290	80.0	60.0
Peatland-Duplex	—	—	—	Warrior	6991	80.0	87.5
Reka 1	5051	5.0	0.2	Wheeler's Thorpe	—	77.5	90.0
Rex	6618	72.5	87.5	White Gatami	920	80.0	72.5
Ricardo	6306	0.2	0.0	Wisconsin Selection	—	67.5	—
Rimpau	2220	45.0	62.5				

uniform rust nurseries. A number of apparently resistant varieties became susceptible. This breakdown in resistance may be due to a number of factors, not the least of which could be a virulent and aggressive physiologic race, or several of them. Such a race or races might be of rare occurrence up to a given time and then become prevalent.

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A PORTABLE BOLL STRIPPER FOR HARVESTING INDIVIDUAL FLAX PLANTS¹

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ABSTRACT

A small boll stripper was designed for harvesting single flax plant selections in the field. This method of harvesting single plants eliminates mixing of seed of different plants; the threshing of the plants is very much simplified; and there is a saving of time in the selecting and threshing operations.

INTRODUCTION

The common method of harvesting individual plants in flax breeding nurseries is to pull the plants by hand and tie all plants from a given plot or family together in a bundle. The plants are later threshed individually. The disadvantage of this method is that, because of the branching nature of the flax plant, the branches become entangled and bolls break off mixing seed from different plants. This boll stripper was developed for stripping and bagging the bolls from the individual plants in the field.

OPERATION

The plant is pulled from the plot by hand and the upper seed-bearing portion is placed in the hopper and the lid lowered (Figure 1). The plant is pulled through the saw teeth which strip the bolls from the stalks. The bolls are funnelled into a small paper bag which sits open on the shelf with the 2-inch tube fitting into it. This leaves both hands free to hold the lid closed and pull the plant through between the teeth. A suggested modification would be to incorporate a foot-operated linkage to hold the cover open for convenience when placing the plant in the hopper. The bag is stapled shut and the plant number written on the bag. The practice at this laboratory has been to thresh the plants by passing the unopened bags through a "roller" thresher.⁽¹⁾

DISCUSSION

The advantages of this device are:

- (1) It eliminates mixing of seed of different plants.
- (2) Threshing of individual plants is very much simplified.
- (3) It saves time in selecting and threshing operations.
- (4) It is very light and portable.
- (5) The bulkiest parts of the plant are left in the field. Thus the bolls from several thousand individual plants can be stored in a relatively small space.
- (6) It is inexpensive and simple to construct. Any competent tinsmith or sheet metal worker should be able to make a duplicate from the information given here.

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FIGURE 1. Portable boll stripper. (Dimensions: *Depth*, 40"; *top measurement*, 12" x 9½"; *bottom*, 18"; *shelf*, 15" x 13")

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EFFECT OF FREQUENCY OF ADMINISTRATION OF CHROMIC OXIDE ON ITS FECAL EXCRETION PATTERN BY GRAZING WETHERS¹

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ABSTRACT

To each of 8 grazing wethers a total of 10 gm. chromic oxide (Cr_2O_3) was administered daily as a single dose or in equal portions twice a day or every 4 hours. Recovery of Cr_2O_3 was determined by total collection of the feces and diurnal excretion patterns were estimated from grab samples taken every two hours. Average recoveries were 101, 95, and 87 per cent for three 4-day trials (4 sheep) and 101 per cent for an 8-day trial (4 sheep). Frequency of administration had little or no effect on recovery.

When the Cr_2O_3 content of grab samples of feces was calculated as per cent of daily average and plotted against time of sampling, the excretion patterns followed the same trend for the same animal on different days. However, although the patterns for the 2 animals dosed twice daily were similar, the 2 animals on once-a-day dosing exhibited distinctly different patterns. Cr_2O_3 content of grab samples varied from 45 to 180 per cent with once-a-day dosing and 65 to 135 per cent with twice-a-day dosing. There was no diurnal trend in the excretion pattern when animals were dosed six times a day.

The length of preliminary period required for average daily Cr_2O_3 recovery to equal intake was found to be 10 days, although average daily recovery reached 90 per cent within 4 days after dosing began.

INTRODUCTION AND LITERATURE REVIEW

The accurate measurement of herbage consumption by freely grazing animals has challenged investigators for many years. Internal and external indicators will, it is hoped, provide a simple technique by which fecal dry matter output and coefficients of digestibility can be obtained simultaneously without the necessity for total fecal collections.

Chromium sesquioxide (Cr_2O_3), commonly called chromic oxide, appears to be the most promising of the external indicators. Barnicoat (1) administered it in 2-gm. gelatin capsules three times daily to 2 wethers fed pasture silage. During a 5-day fecal collection period, following an 8-day preliminary period, he recovered an average of 91 per cent of the daily dose. Crampton and Lloyd (2) used 4 wethers in digestion stalls to study the recovery of Cr_2O_3 . Three trials were conducted. Each comprised a 6-day preliminary period and a 13-day collection period. All animals received chopped timothy hay as the roughage. Two wethers received the Cr_2O_3 in a special pelleted form (pellets formed of Cr_2O_3 mixed with a small amount of concentrate and molasses). The other 2 wethers were administered the indicator by mixing it with larger amounts of concentrate, amounting to 35 per cent of the total ration. Each ration was fed both once and twice per day. Average fecal recovery of the indicator was 98.5 per cent when fed mixed in the meal but only 86.0 per cent when fed in the pelleted form.

¹ Contribution from Division of Animal Husbandry, Experimental Farms Service, Ottawa, and Contribution No. 293 from Chemistry Division, Science Service, Ottawa.

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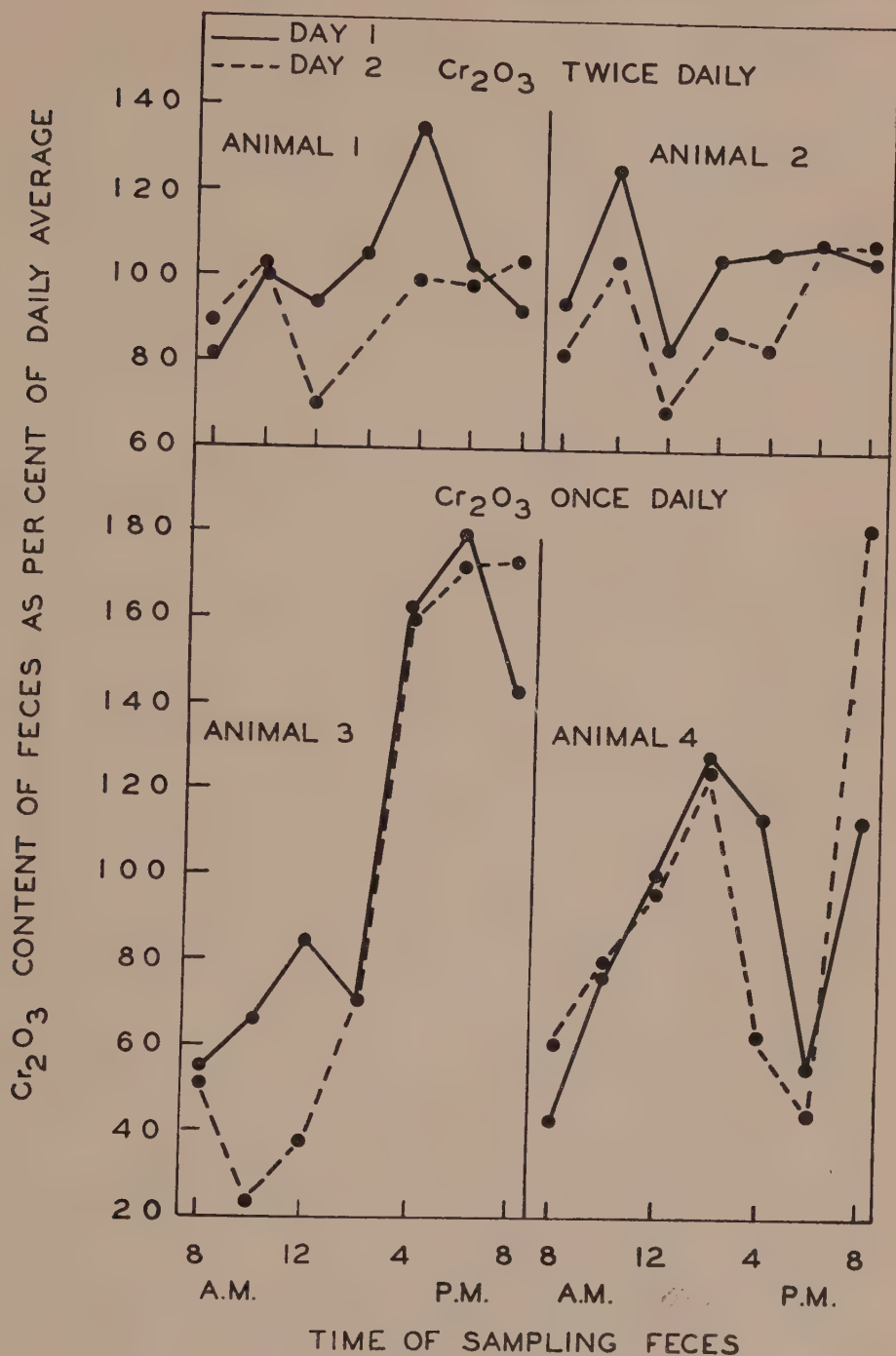


FIGURE 1. Diurnal excretion cycle of chromic oxide by grazing wethers (Trial III).

Kane *et al.* (4) reported that, when Cr_2O_3 was fed in the concentrate part of the ration to dairy cows, the indicator was not uniformly distributed in the feces but varied in a more or less regular diurnal pattern. Hardison and Reid (3) made similar observations with grazing steers dosed once daily with a gelatin capsule containing 15 gm. Cr_2O_3 . The fecal concentration of Cr_2O_3 varied within a 24-hour period from 55 to 180 per cent of the daily average. Because of the apparent uniformity of the excretion pattern, however, the authors were able to use partial fecal collections, taken at specific hours, to estimate the dry matter output.

Before this technique could be applied to pasture studies with sheep it was necessary to determine the excretion of Cr_2O_3 by this species. Reported herein are results of studies which relate frequency of dosing with chromic oxide to recovery and diurnal excretion pattern.

EXPERIMENTAL PROCEDURE

Three trials (I, II, and III) were conducted during the summer of 1953 with 4 yearling wethers (No. 1 to 4) grazing on an experimental pasture. The animals were restricted by hurdles to an area sufficient to provide a half-day's grazing and the hurdles were moved morning and evening. This procedure was followed to induce complete consumption of the herbage which was required by another experiment being carried out concurrently. Trial IV was carried out in the laboratory during the same season. In this trial 4 wethers were confined to digestion crates and fed grass which was clipped daily. Trial V was conducted with 4 yearling wethers (No. 5 to 8) in the spring of 1954 as soon as sufficient herbage was available for continuous grazing. These sheep were permitted to graze freely over the entire experimental area. The herbage consumed during each period is described in Table 1.

Cr_2O_3 was administered in gelatin capsules with the aid of a balling gun. For Trials I, II, and III, the indicator was given to the grazing sheep for at least 10 days before the first fecal collection of Trial I, and during the following 30 days both during the experimental trials and in periods between these three trials. A preliminary period of 4 days and 10 days was allowed for Trials IV and V, respectively. The daily dosing schedule is given in Table 1.

Total fecal collections from the grazing animals were made by using collection bags and harnesses. The bags were emptied according to the schedule outlined in Table 1. In Trials I to IV, the feces were sampled on an individual animal basis. In Trial V the collections for the two animals on each dosing treatment were composited and samples of the composite collection's were taken for analysis. Collections were made for a total of 18 days (10 days preliminary and 8 days grab-sampling) beginning when the first capsule was given.

"Grab" samples, *i.e.*, partial fecal collections, were obtained by emptying the collection bags every 4 hours during the day in Trial III. During the first 2 days, collections were started at 6 a.m., and for the next 2 days at 8 a.m., according to the schedule in Table 1. By combining the data from the first and third, and second and fourth days, knowledge

TABLE 1. DETAILS OF EXPERIMENTAL PROCEDURE

Trial No.	Feces Sampling Schedule	Length of Collection Periods	Animal No.	Cr ₂ O ₃ Dosing Schedule (Each animal received 10 gm. Cr ₂ O ₃ /day)
I (Field)	Bags emptied twice daily. Composited for each animal on a 4-day basis.	4 days	1	5 gm. 8 a.m.
			2	5 gm. 4:30 p.m.
			3	10 gm. 8 a.m.
			4	
II (Field)	Bags emptied daily. Composited for each animal on daily basis.	4 days	1	5 gm. 8 a.m.
			2	5 gm. 4:30 p.m.
			3	10 gm. 8 a.m.
			4	
III (Field)	Bags emptied every 4 hours and overnight. <i>Day</i> 1 } <i>a.m.</i> <i>p.m.</i> 2 } 6, 10 2, 6, 10 3 } 4 }	4 days	1	5 gm. 8 a.m.
			2	5 gm. 4:30 p.m.
			3	10 gm. 8 a.m.
			4	
IV (Laboratory)	Laboratory fecal collections composited for each animal every 5 days.	10 days (two 5-day sub-periods)	1 2 3 4	5 gm. 8 a.m. 5 gm. 4:30 p.m.
V (Field)	Bags emptied twice daily during preliminary period and every 2 hours 8 a.m. to 8 p.m. during grab sampling period. 8 a.m. collection = overnight excretion.	10 days' preliminary 8 days' grab sampling	5	5 gm. 8 a.m.
			6	5 gm. 4 p.m.
			7	1-666 gm. every 4 hr.
			8	4 and 8 a.m., 12 noon, 4 and 8 p.m. and 12 midnight.

I — Herbage 10-15" high, heading, 40% timothy, 30% Kentucky blue, 20% white clover, 10% dandelions.
 II — Herbage same as for Trial I, except timothy headed but still pre-bloom.
 III — Herbage second growth, similar in composition to that for Trial I. Leaf tips dried by drought.
 IV — Herbage second growth used canary grass, 6-8" high, very leafy. Freshly cut each day.
 V — Herbage similar in composition to that for Trial I. Early spring growth about 4" high.

of the fecal concentration of Cr_2O_3 in samples taken at an average time of 8 a.m., 10 p.m., 12 noon, 2 p.m., 4 p.m., 6 p.m., 8 p.m. and overnight was obtained. This sampling schedule was adopted to avoid disturbing the animals unduly and to provide sufficient fecal material for other chemical analyses in addition to Cr_2O_3 . Grab-samples were also taken for 8 days during Trial V, as outlined in Table 1.

The fecal samples were dried at 100°C . and finely ground prior to analysis. Cr_2O_3 determinations were made by a procedure to be described in a subsequent publication.

RESULTS AND DISCUSSION

Recovery of Chromic Oxide

The fecal recovery of Cr_2O_3 for Trials I to IV is given in Table 2. The 4-day averages for all animals for Trials I and II were 101 and 94 per cent respectively. However, the average for Trial III was lower at 87 per cent. This was chiefly due to the low recovery from Animal 3 throughout the collection period and from all animals on the last day of the period. There was no marked difference in the average recovery between the once- and twice-daily dosing treatments. Recovery of the indicator in Trial IV (laboratory) was very satisfactory for the second 5-day period but rather low—93 per cent—during the first 5 days. This low recovery was probably caused by the very short, 4-day, preliminary period.

Table 3 shows the fecal recovery of Cr_2O_3 during Trial V. The maximum deviation from 100 per cent recovery was not more than 2 per cent for the 8-day averages, regardless of the frequency of dosing. The deviations were slightly higher for the 4-day averages. However, it is evident

TABLE 2.—RECOVERY OF CHROMIC OXIDE FROM THE FECES OF WETHERS (PER CENT OF AMOUNT FED)

Trial	Date 1953	Frequency of Administration				Av.
		Twice daily		Once daily		
		Animal 1	Animal 2	Animal 3	Animal 4	
I (Field)	July 25-29 Av.	99	95	108	100	101
II (Field)	July 3	84	96	129	77	97
	July 4	123	122	65	115	106
	July 5	114	65	95	90	91
	July 6	102	72	66	92	83
	Av.	106	89	89	94	94
III (Field)	July 21	96	79	73	94	86
	July 22	113	99	84	104	100
	July 23	85	96	94	86	90
	July 24	79	78	67	63	72
	Av.	93	88	80	87	87
IV (Laboratory)		115	78	96	84	93
		99	95	108	100	101
	Av.	107	86	102	92	97

TABLE 3.—RECOVERY OF CHROMIC OXIDE FROM THE FECES OF GRAZING WETHERS IN TRIAL V (PER CENT OF AMOUNT FED)

Date (1954)	Frequency of Administration		Av.
	Twice daily	Six times daily	
May 25	104	112	108
May 26	116	103	110
May 27	97	90	93
May 28	92	109	100
4-day Av.	102	104	103
May 29	98	109	104
May 30	97	100	98
May 31	92	95	94
June 1	108	96	102
4-day Av.	99	100	100
8-day Av.	100	102	101

that the maximum deviation for any period of 4 successive days which can be selected from these data does not exceed ± 6 per cent and is usually of the order of 2 to 3 per cent.

With the exception of Trial III the recoveries of Cr_2O_3 in these experiments were considerably higher than those reported by Barnicoat (1) using gelatin capsules, and by Crampton and Lloyd (2) from the wethers fed Cr_2O_3 in pellet form.

Daily recoveries in Trial V were more uniform than those found for Trials II and III. This may have been because the animals in Trial V were permitted to graze freely and perhaps their intake and excretion of dry matter was more uniform than for the earlier trials, where the animal's grazing was limited according to the judgement of the field operator.

Diurnal Excretion Pattern

The fecal concentration of Cr_2O_3 obtained for each grab-sample in Trials III and V was converted to a percentage of the corresponding daily average as follows:

$$\frac{\text{Cr}_2\text{O}_3 \text{ concentration in grab-sample (\% of daily av.)}}{\text{Cr}_2\text{O}_3 \text{ (mg./gm. dry feces) in grab-sample}} = \frac{\text{Cr}_2\text{O}_3 \text{ (mg./gm. dry feces) in daily av.}}{\text{Cr}_2\text{O}_3 \text{ (mg./gm. dry feces) in daily av.}} \times 100$$

These percentages for Trial III are plotted against time in Figure 1 and show that the fecal concentration of Cr_2O_3 varies in definite diurnal patterns. These variations in fecal concentration are more extreme when the indicator is administered only once daily, varying from 45 to 180 per cent for the once-daily treatment compared to 65 to 135 per cent for

TABLE 4.—VARIANCE ANALYSIS OF CONCENTRATION OF CHROMIC OXIDE (PER CENT OF DAILY AVERAGE) IN GRAB SAMPLES, TRIAL III

Source of variance	D.F.	Mean squares	
		Once daily	Twice daily
Total	27	—	—
Animals	1	999.4	2.0
Days	1	99.3	925.2**
Time	6	5062.7**	3177.8**
A X D	1	307.2	1.2
A X T	6	4289.5**	117.1
D X T	6	706.5	153.0
Error	6	291.4	40.5

**Significant ($P < 0.01$)

TABLE 5.—VARIANCE ANALYSIS OF CONCENTRATION OF CHROMIC OXIDE IN FECAL GRAB SAMPLES, TRIAL V

Source of variance	D.F.	Mean Squares					
		% of daily av.		% of 8-day av.		Mg./gm. dry feces	
		2/day	6/day	2/day	6/day	2/day	6/day
Total	55	—	—	—	—	—	—
Time	7	2310.7**	19.1	2295.8**	19.3	150.2**	1.7
Day	6	101.3	109.8	1454.7**	748.7	69.9**	47.3**
Error	42	90.1	44.4	94.4	44.3	5.3	3.3

**Significant ($P < 0.01$)

TABLE 6.—CONCENTRATION OF CHROMIC OXIDE, MG. PER GM. DRY FECES, IN GRAB SAMPLES, TRIAL V.

(Av. of 8-day Period)

Cr ₂ O ₃ treatment	Time						
	A.M.			P.M.			
	8	10	Noon	2	4	6	8
6/day	27.17	26.97	26.47	27.92	27.01	27.45	26.91
2/day	26.62	21.23	17.52	15.95	22.22	26.21	26.08

the twice-daily group. The diurnal excretion patterns of the 2 animals dosed daily were similar, but the 2 animals on the once-a-day regime each exhibited a distinctly different pattern. However, similar patterns were obtained on different days for a given animal. Twice-daily dosing reduced the animal variation in excretion patterns. This effect, is clearly indicated

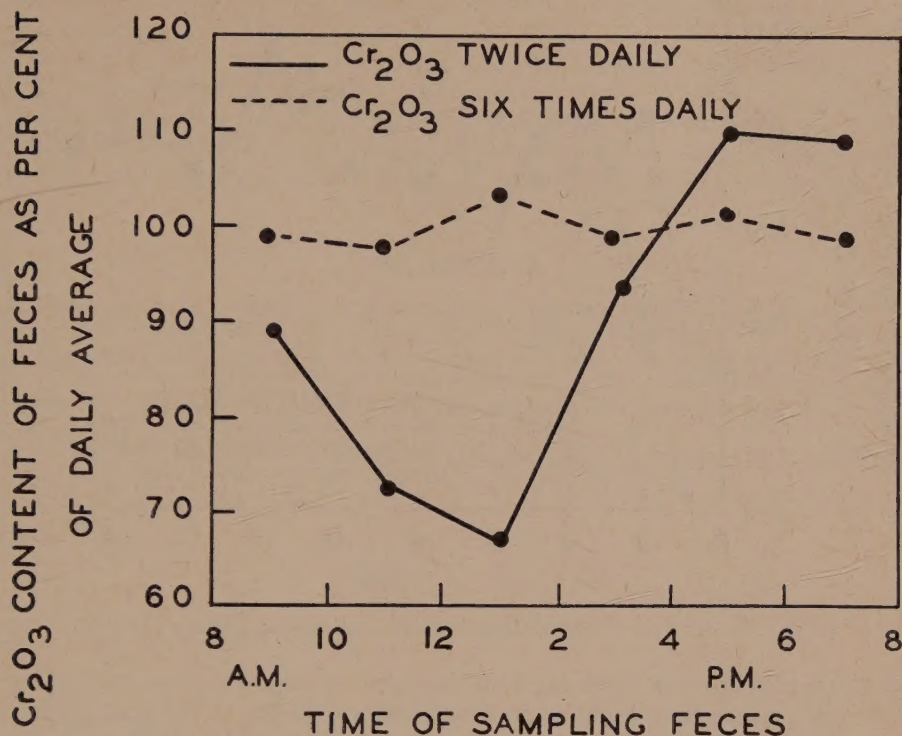


FIGURE 2. Diurnal excretion cycle of chromic oxide by grazing wethers (Trial V).

in the highly significant mean squares for the animal \times time interaction for the once-daily treatment given in Table 4. Highly significant differences were found for both days and time for the twice-daily treatment (Table 4).

It is of interest to note that the excretion pattern between 8 a.m. and 8 p.m. for the once-daily Cr_2O_3 treatment was the opposite of that observed for similarly treated steers at this Farm*(3). In general, when the fecal concentration of Cr_2O_3 was the highest for the sheep, it was lowest for steers.

The diurnal variation in the fecal excretion of Cr_2O_3 in Trial V is illustrated in Figure 2. The graph shows that the animals on the twice-per-day dosing schedule excreted the indicator in a diurnal pattern similar to the comparable treatment in Trial III. However, the animals on six-times-daily treatment excreted the indicator in a very uniform manner; the maximum 8-day average deviation from 100 per cent was only 3 per cent and usually was much less.

An analysis of variance of the Cr_2O_3 concentration in the grab-samples for Trial V expressed as (a) percentage of daily average, (b) percentage

*Unpublished data.

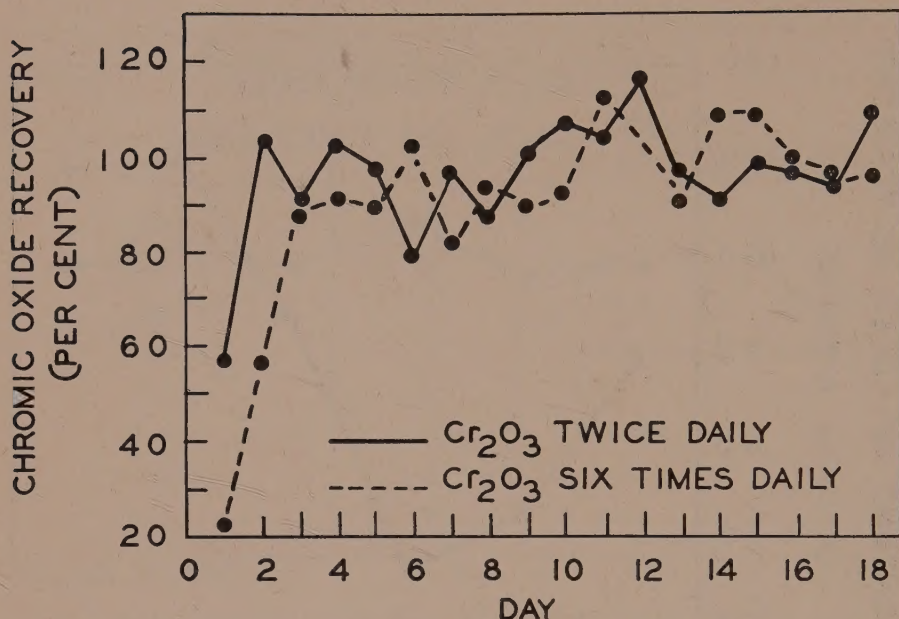


FIGURE 3. Daily recoveries of chromic oxide from grazing wethers (Trial V).

of 8-day average and (c) as mg. Cr_2O_3 per gm. dry feces is given in Table 5. The table shows that highly significant time variations in the fecal concentration of Cr_2O_3 were found for the twice-daily treatment but those for six-times-daily treatment were not significant regardless of how the concentration was expressed. Variations in concentration between days were not significant when the data were expressed as a percentage of the daily average but were highly significant when expressed as a percentage of 8-day average or as mg. per gm. dry feces. These data mean that grab-samples of feces may be taken at any time during the day from 8 a.m. to 8 p.m. when wethers are dosed six times daily. However, since the analysis of variance showed that the fecal concentration of the indicator will vary from day to day, grab-samples must be taken every day to obtain reliable estimates of dry matter output.

A worthwhile (50 per cent) and significant ($P = 0.025$) reduction in grab-sampling error occurred when animals were dosed six times instead of twice daily.

Table 6 shows the 8-day average fecal concentration of Cr_2O_3 expressed as mg. per gm. dry feces. Expressed on this basis, concentration follows the same general diurnal trend for both dosing treatments as in Figure 2, where the data are expressed as percentages. These patterns, therefore, can be determined without making total feces collections which are necessary for calculating the percentage values, with a consequent saving in labour.

Preliminary Period

The daily recoveries of Cr_2O_3 during the 18 days of Trial V are illustrated in Figure 3. Recovery of the indicator in the feces of the wethers

dosed twice daily reached 100 per cent within 48 hours but the group on the six-times-daily dosing treatment required 6 days to reach this level. This represented more rapid passage of the indicator than was reported by Crampton and Lloyd (2) for wethers on dry feed. However, in general, average recoveries for all animals were below 100 per cent until the tenth day. These data indicate that, under conditions prevailing in this experiment, a preliminary period shorter than 10 days would be inadvisable.

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